Aging & Rehabilitation

An Interdisciplinary Research Seminar Series

UNIVERSITY OF FLORIDA

Sponsors

Department of Veteran Affairs

- Rehabilitation Outcomes Research Center (RORC)
- Brain Rehabilitation Outcomes Research Center (BRRC)
- Geriatric Research, Education, and Clinical Center (GRECC)

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- Department of Aging and Geriatric Research

UF College of Public Health and Health Professions

- Brooks Center for Rehabilitation Studies

UF College of Liberal Arts and Sciences

- Center for Gerontological Studies

UF McKnight Brain Institute

UF College of Nursing

Schedule

- August 29th, 2005 – May 22nd, 2006
- Mondays, 12:00 – 1:00
- HPNP Room – G103

CYBER SEMINAR VENUES

- VA RORC, Conference Room, Suite 350
- VA BRRC, VA Nursing Home, Room 271-12
- UF Brooks Center Conference Room, Jacksonville

Themes

- Basic Science (C. Leeuwenburgh)
- Clinical Science (R. Beyth)
- Outcomes / Health Policy (E. Andresen)
- Behavioral and Social Research (M. Marsiske)
- Cutting Edge / New Research (T. Foster/ J. Aris)

Microglial Activation and Neuroinflammation

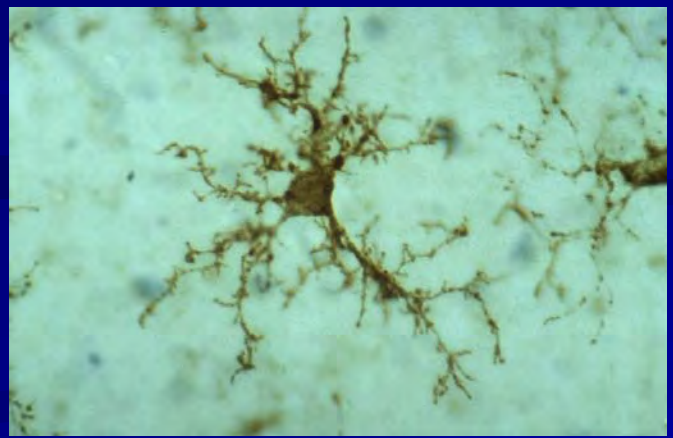
Wolfgang J. Streit, Ph.D.
Department of Neuroscience

Outline and Objectives

- Learning Objectives
- 1. Understand that microglial cells comprise the brain's immune system.
- 2. Understand that microglia are the primary cells engaged in the brain's response to injury.
- 3. Realize that microglial cells are subject to aging and that this may contribute to the development of aging-related neurodegenerative disease.

Definitions

- Microglia (microglial cells): a population of non-neuronal (glial) cells in the CNS. Microglia are the brain's representatives of the immune system. They share some characteristics with white cells of the blood, but have specially evolved to live in the brain's unique microenvironment.



Definitions, cont'd

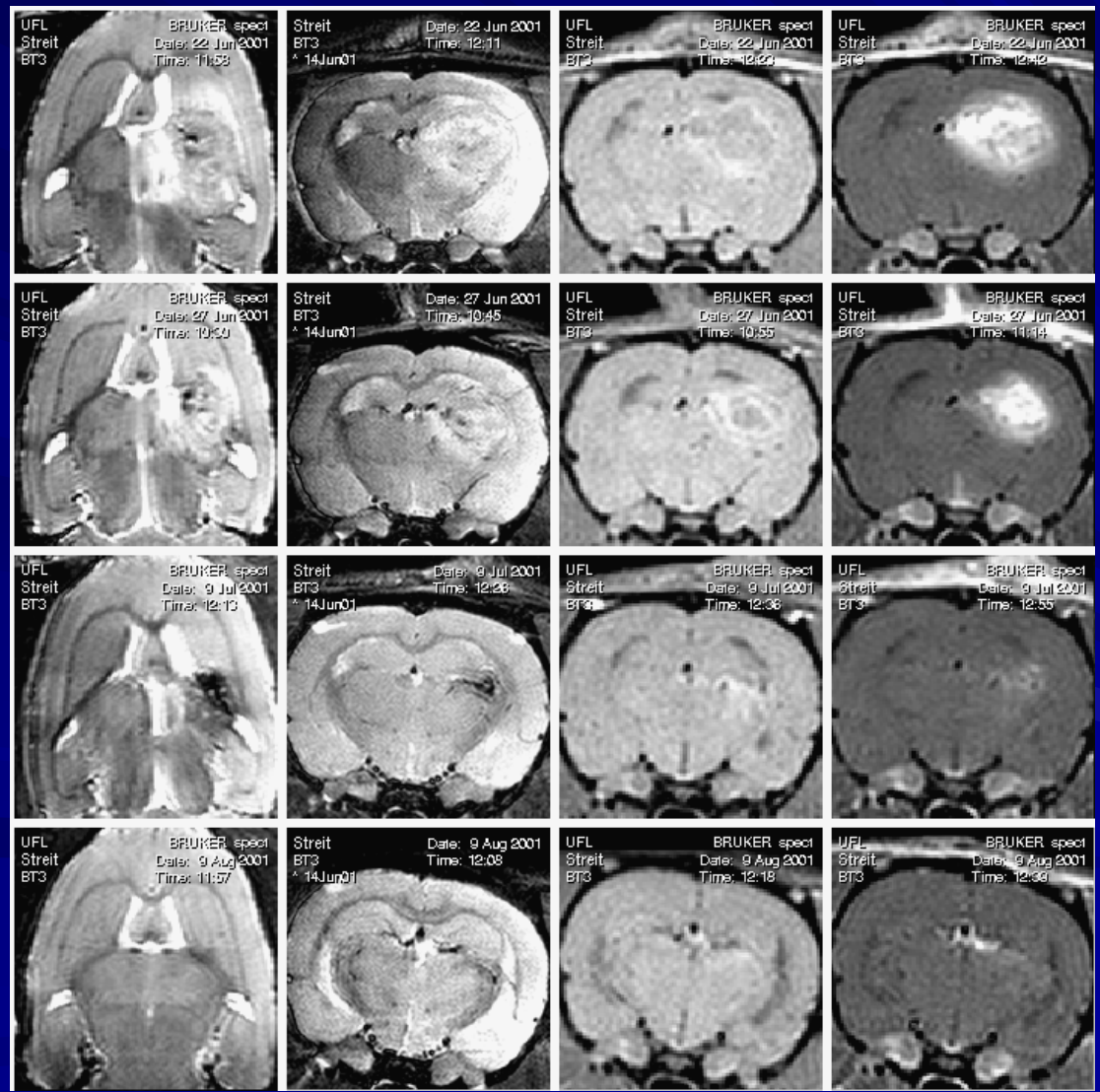
- Inflammation: The reaction of living tissues to all forms of injury.
- Acute *versus* Chronic (beneficial and wound healing *versus* detrimental and pathological)
- Neuroinflammation: The reaction of living nervous tissue to all forms of injury.

Is the brain really “immunologically privileged”?

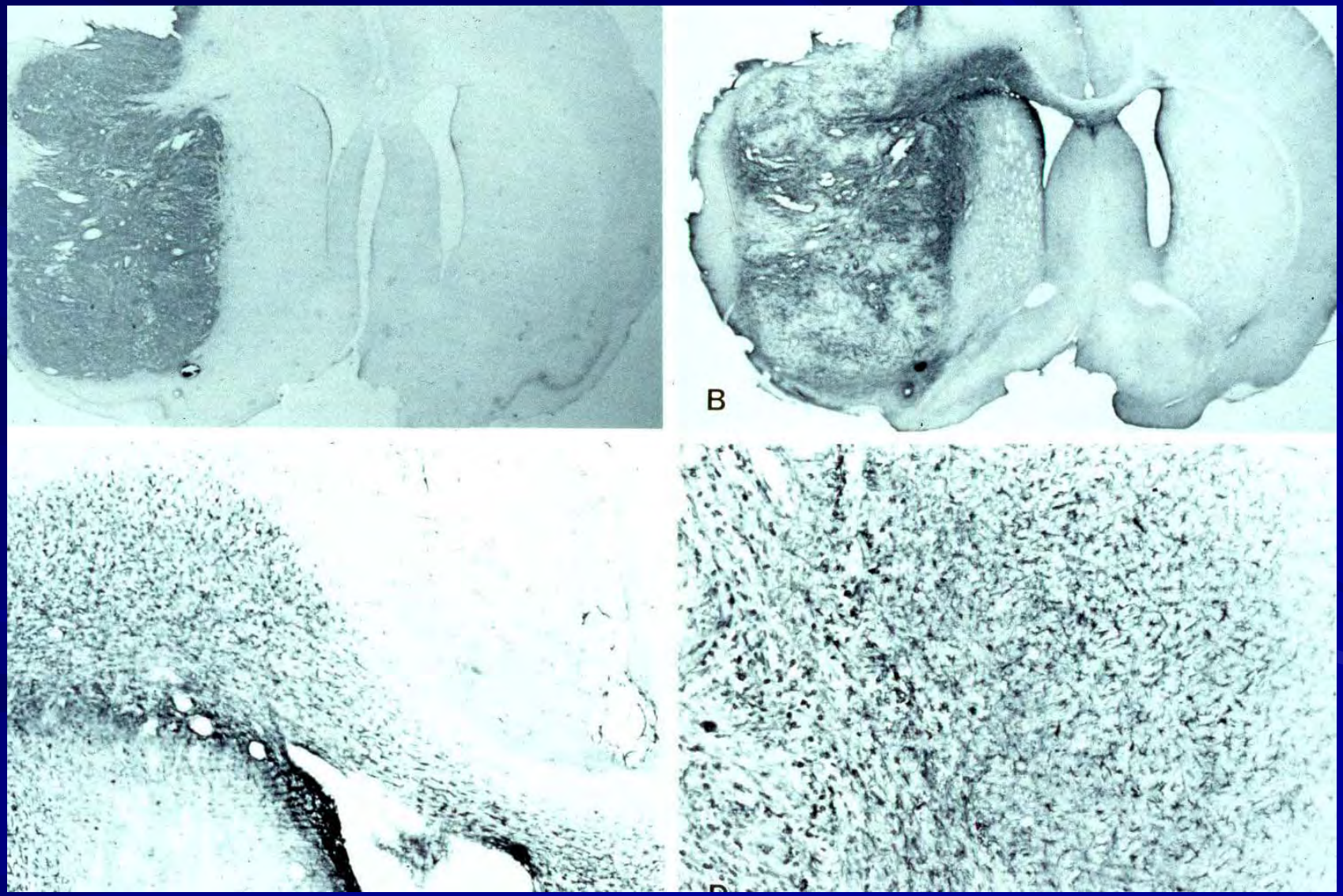
- Better graft survival
- No lymphatic drainage
- No MHC antigens
- No lymphocytes

- Blood brain barrier (BBB)

Allogeneic brain tumors (RG2 glioma) are subject to spontaneous rejection.



Brain tumors are infiltrated by microglial cells and peripheral leukocytes.



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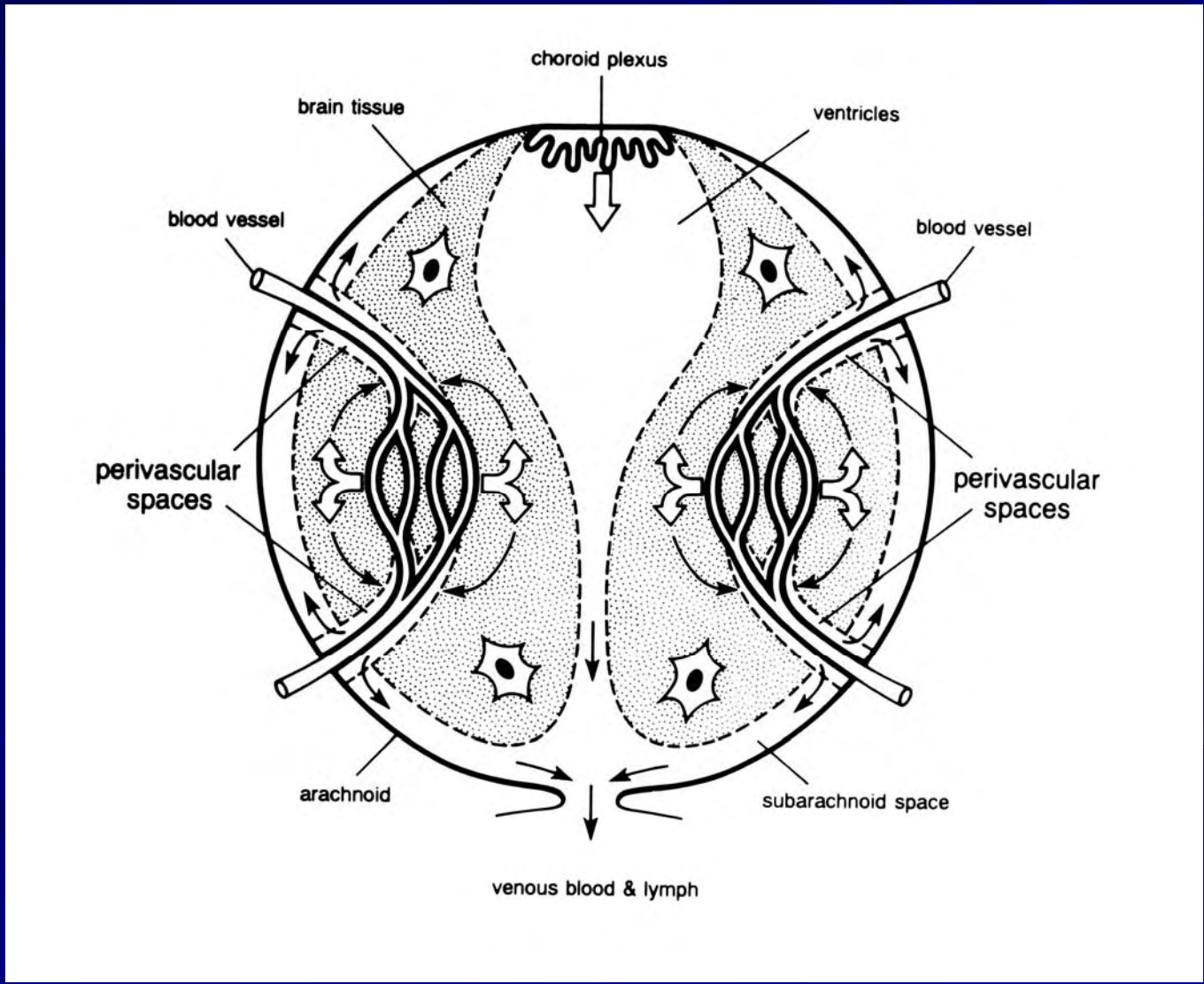
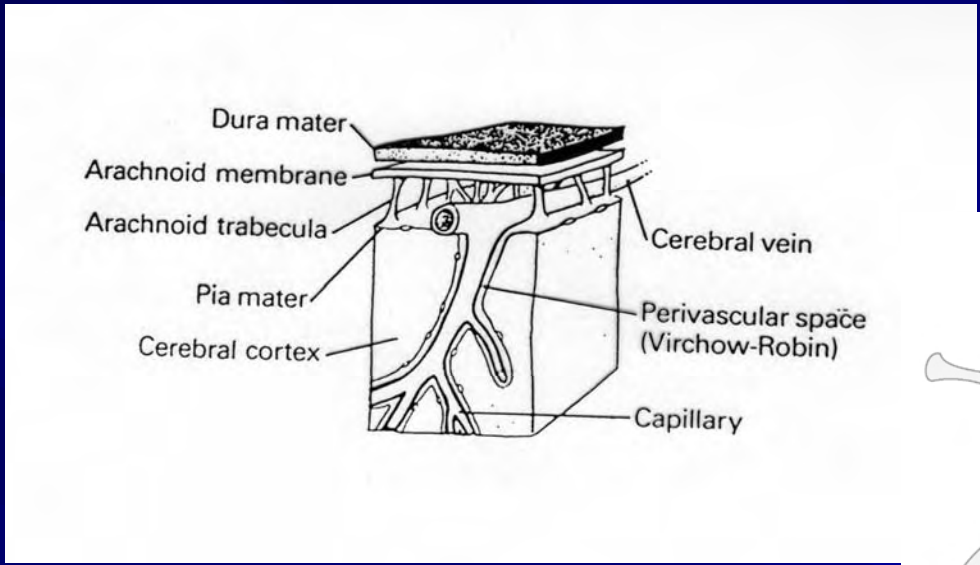
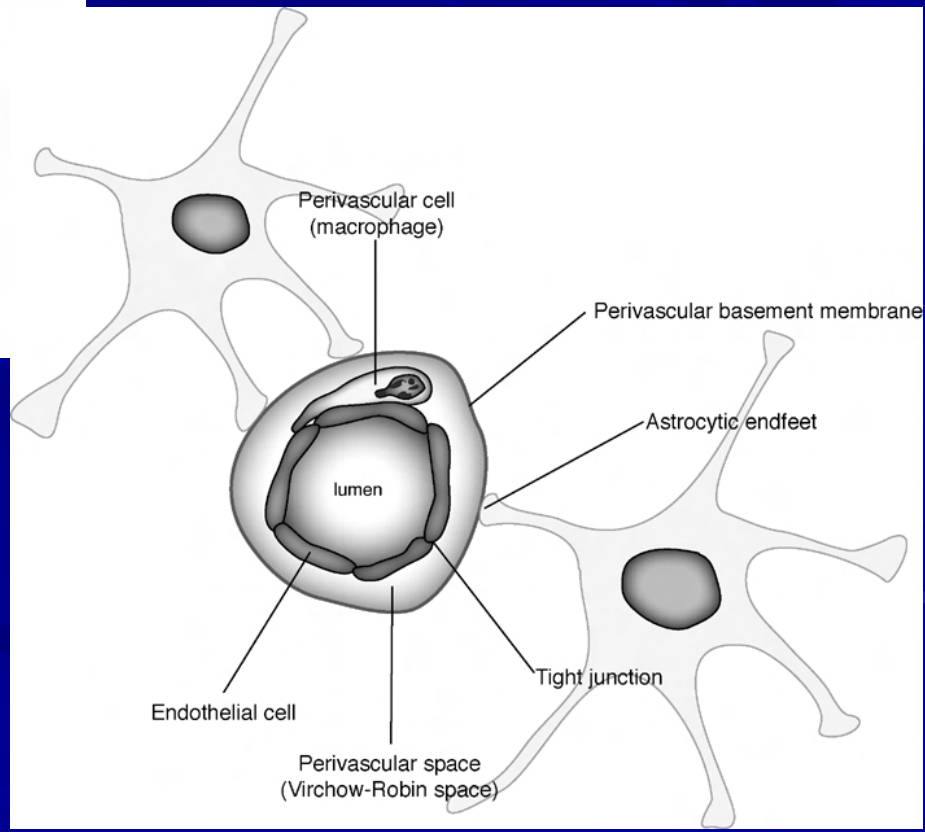
Interstitial Fluid Turnover



Cserr et al. Brain Pathol. 2:269-276 (1992)

The perivascular (Virchow-Robin) space: neuro-immune interface





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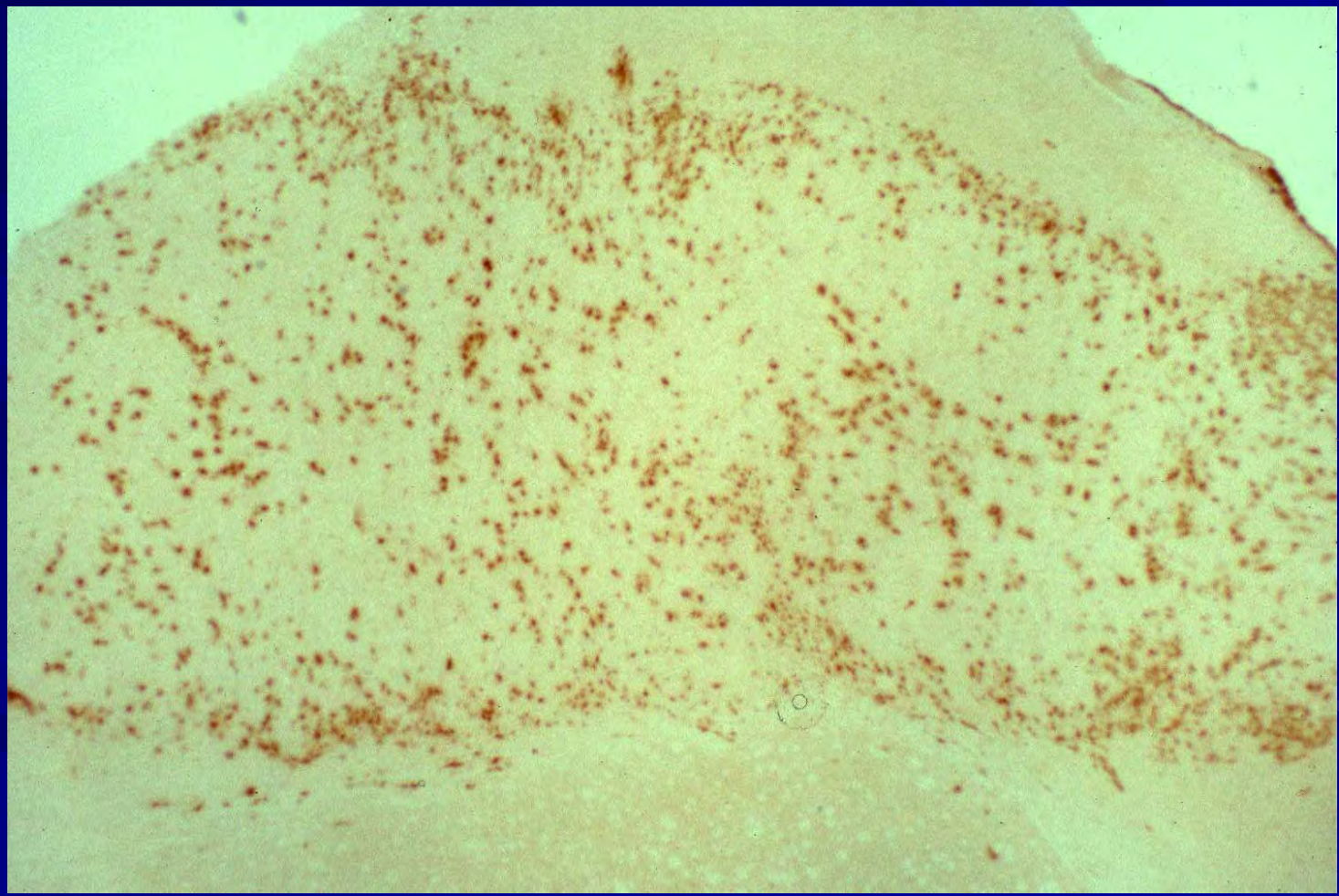
The expression of major histocompatibility complex (MHC) antigens is increased in the injured CNS.

Example 1:

Brain tumors

OX-6 for MHC II



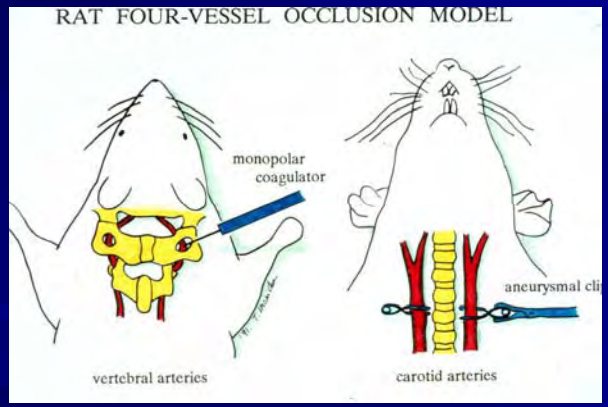
The expression of major histocompatibility complex (MHC) antigens is increased in the injured CNS.

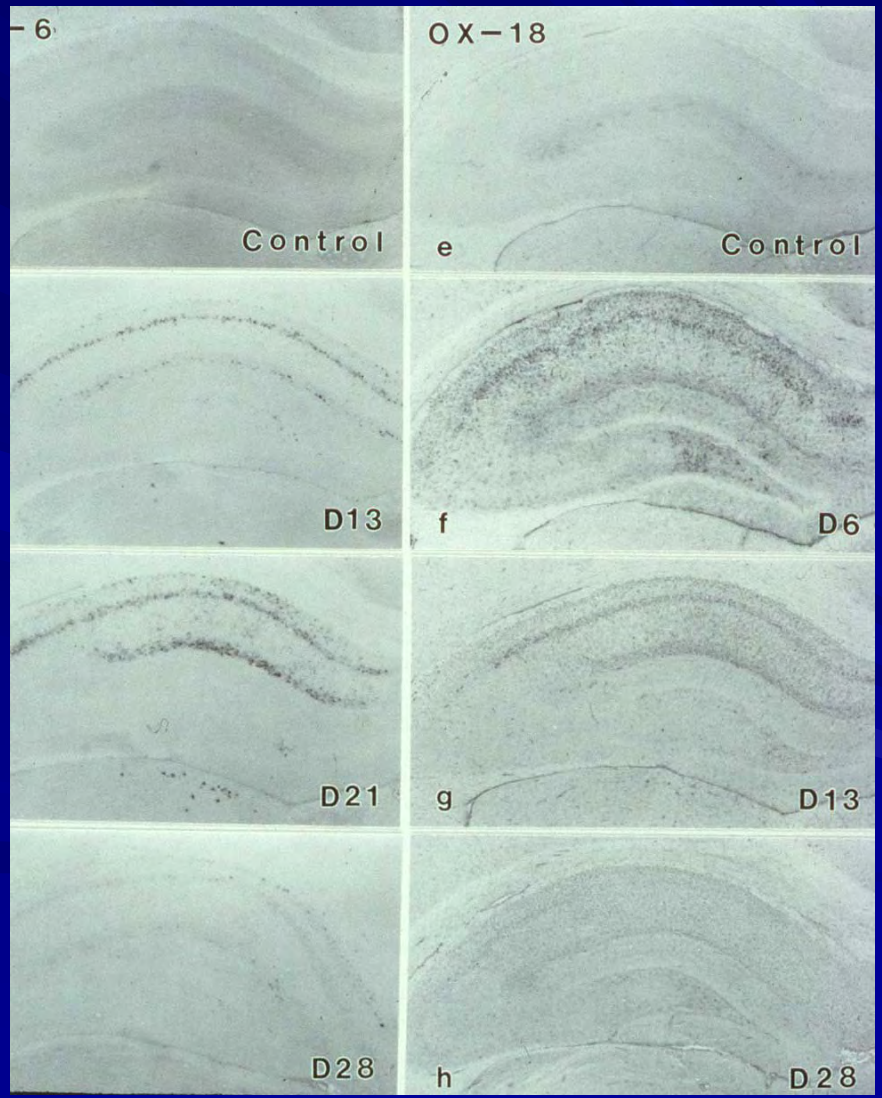
Example 2:

Global forebrain ischemia

OX-6 for MHC II

OX-18 for MHC I



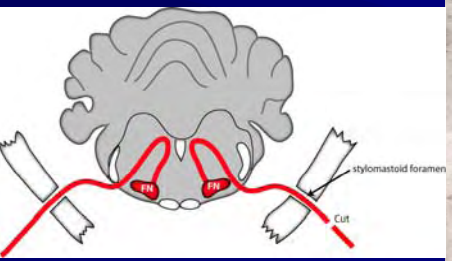


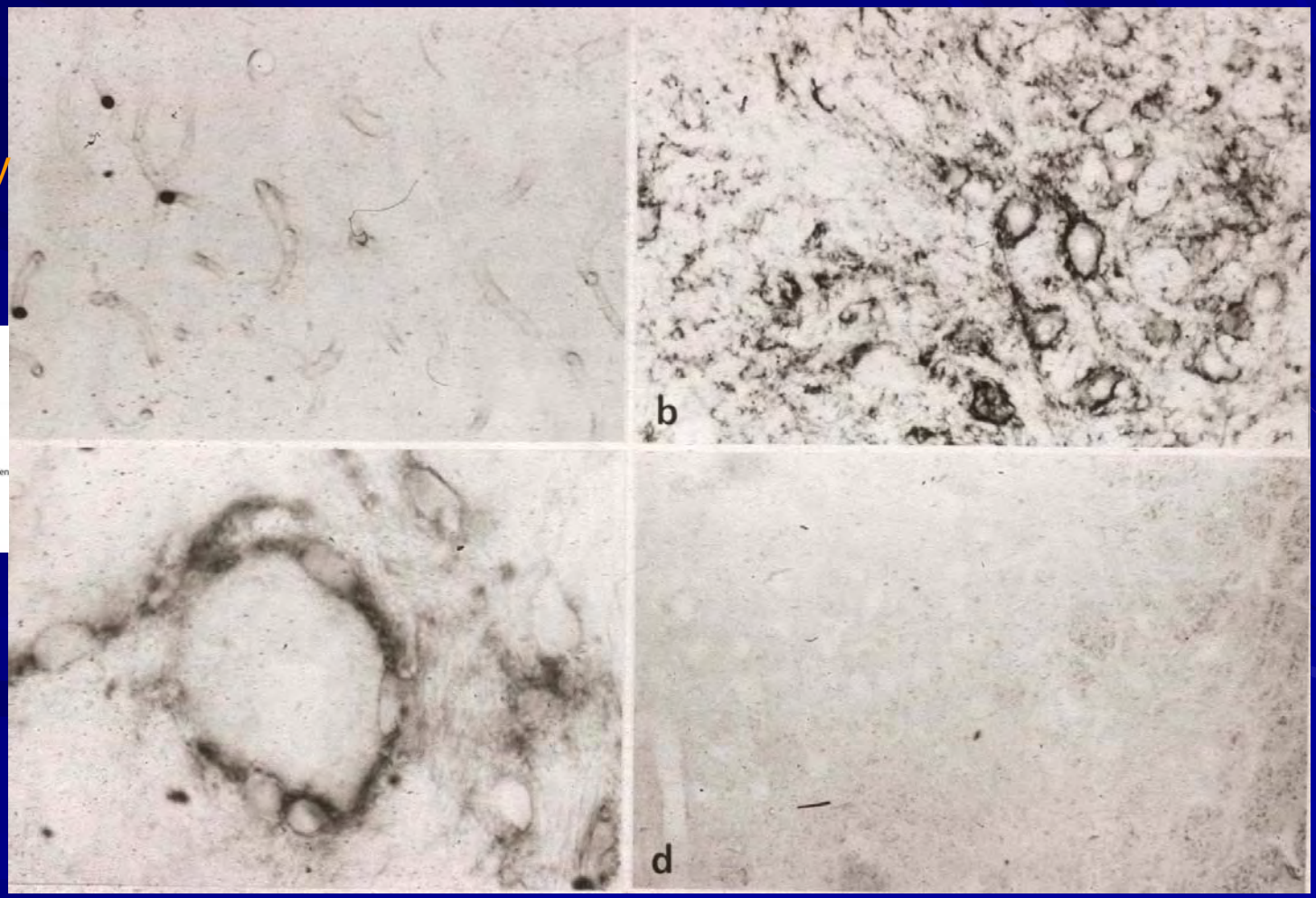
The expression of major histocompatibility complex (MHC) antigens is increased in the injured CNS.

Example 3:

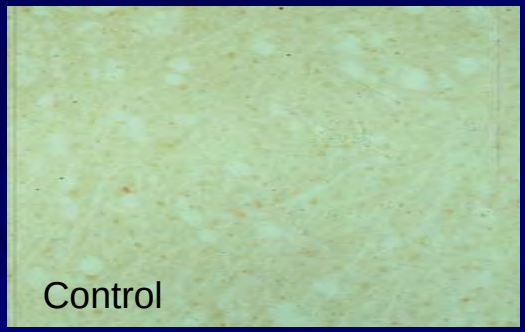
Facial N. axotomy

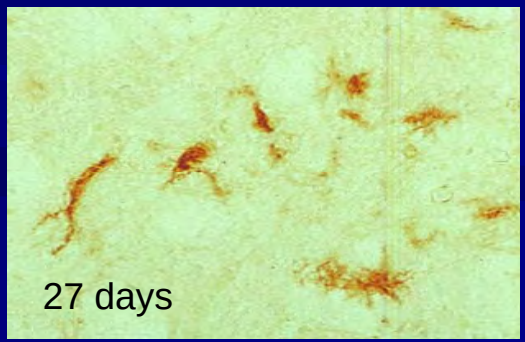
OX-18 for MHC I

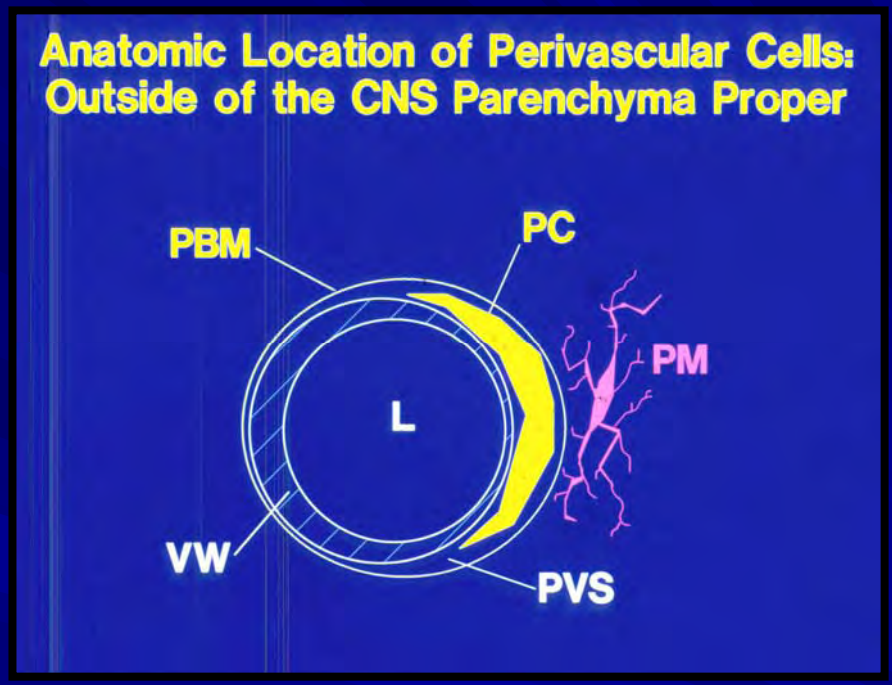




MHC class II antigens are found on both microglia and perivascular cells.







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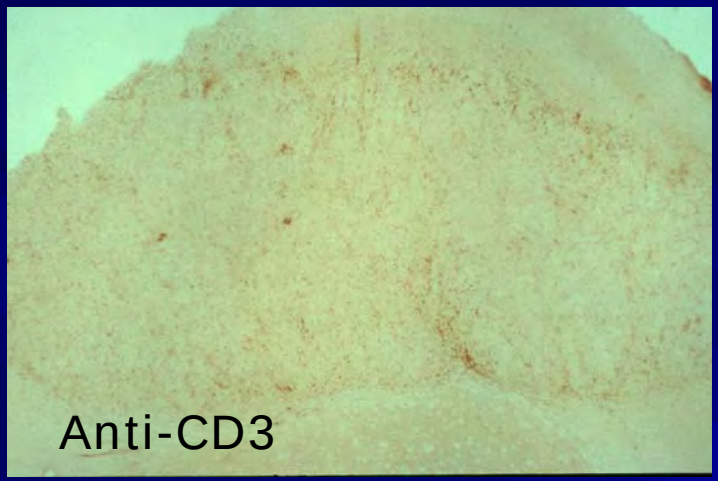
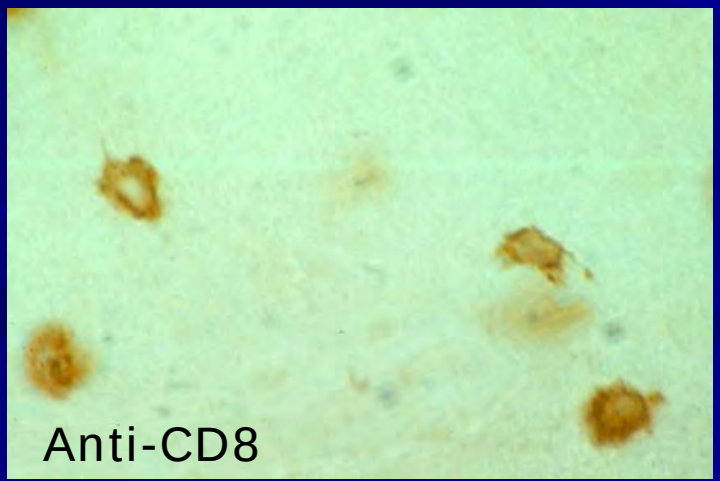
- Blood brain barrier (BBB)

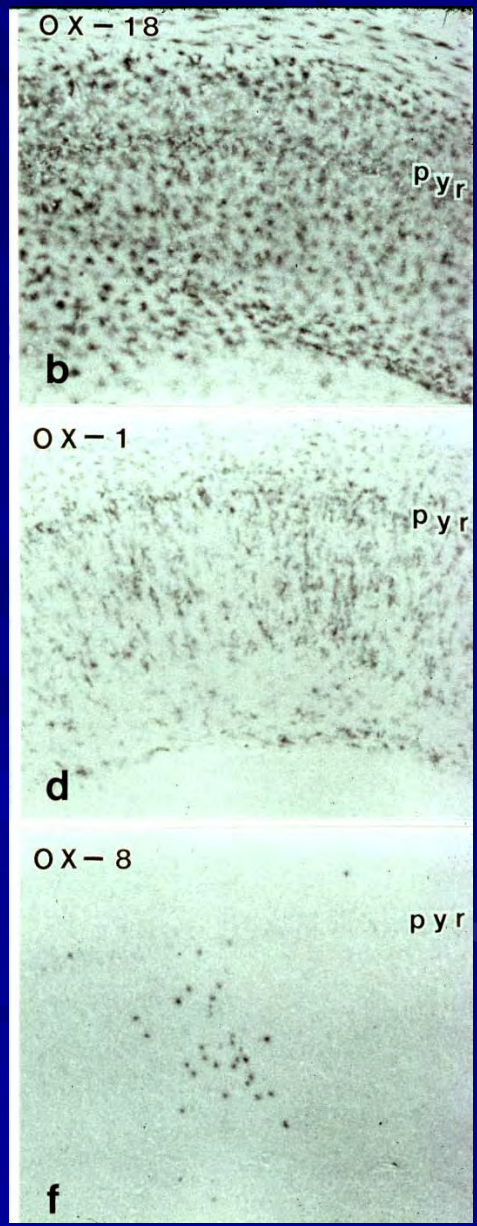
Lymphocytes infiltrate during CNS pathology.

In gliomas:

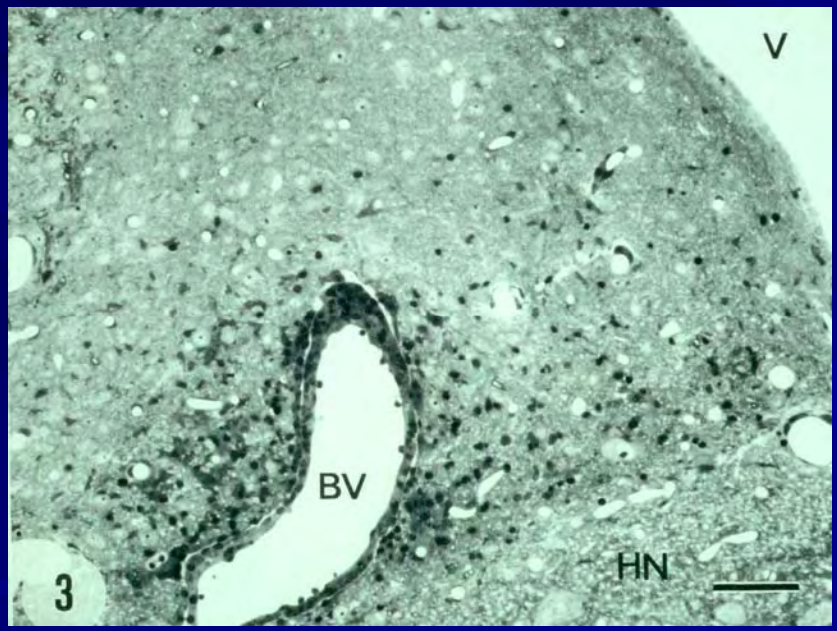


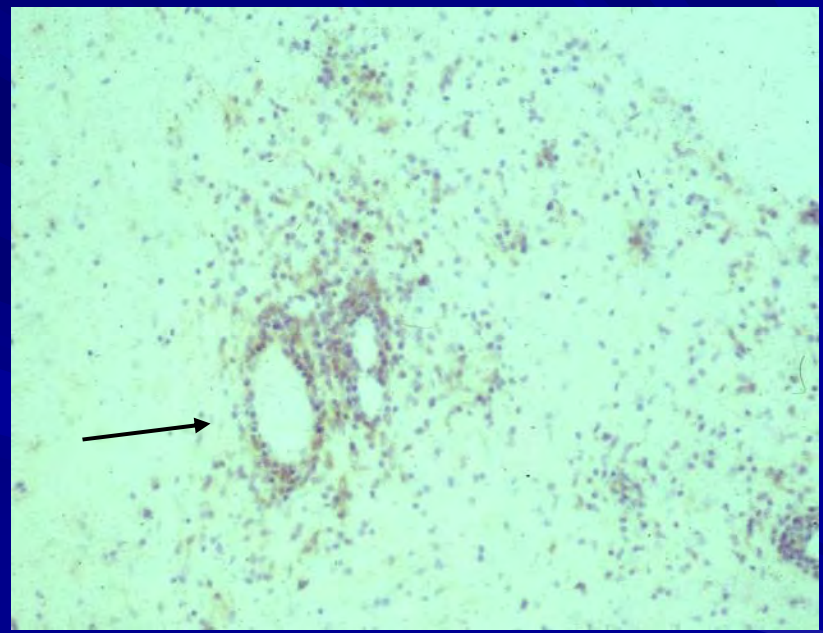


During ischemia:



Lymphocytes infiltrate during CNS pathology, especially during autoimmune disease, resulting in perivascular accumulations.





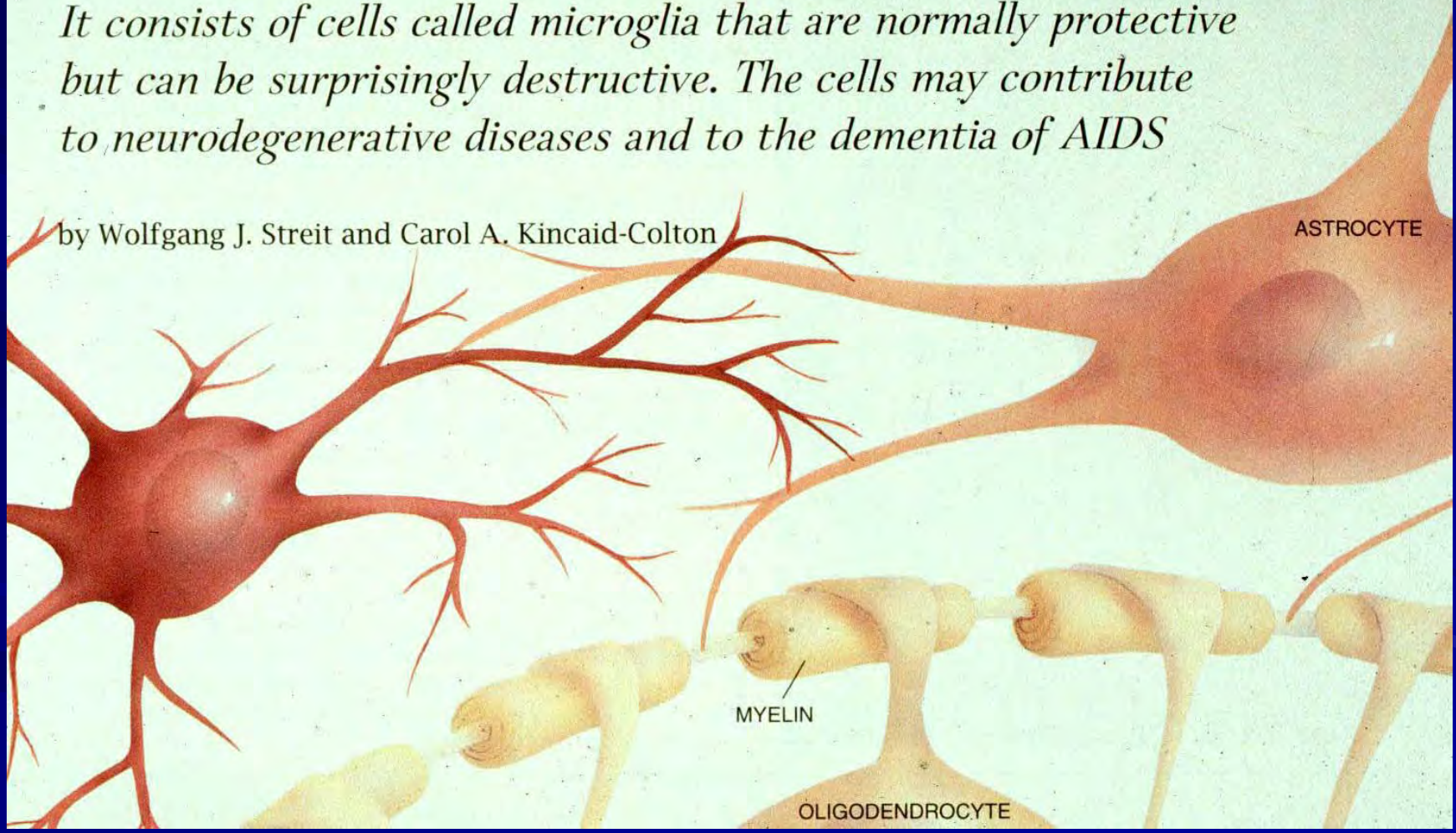
The brain’s immune privilege is limited.

And defined primarily by the blood brain barrier.

The Brain's Immune System

It consists of cells called microglia that are normally protective but can be surprisingly destructive. The cells may contribute to neurodegenerative diseases and to the dementia of AIDS

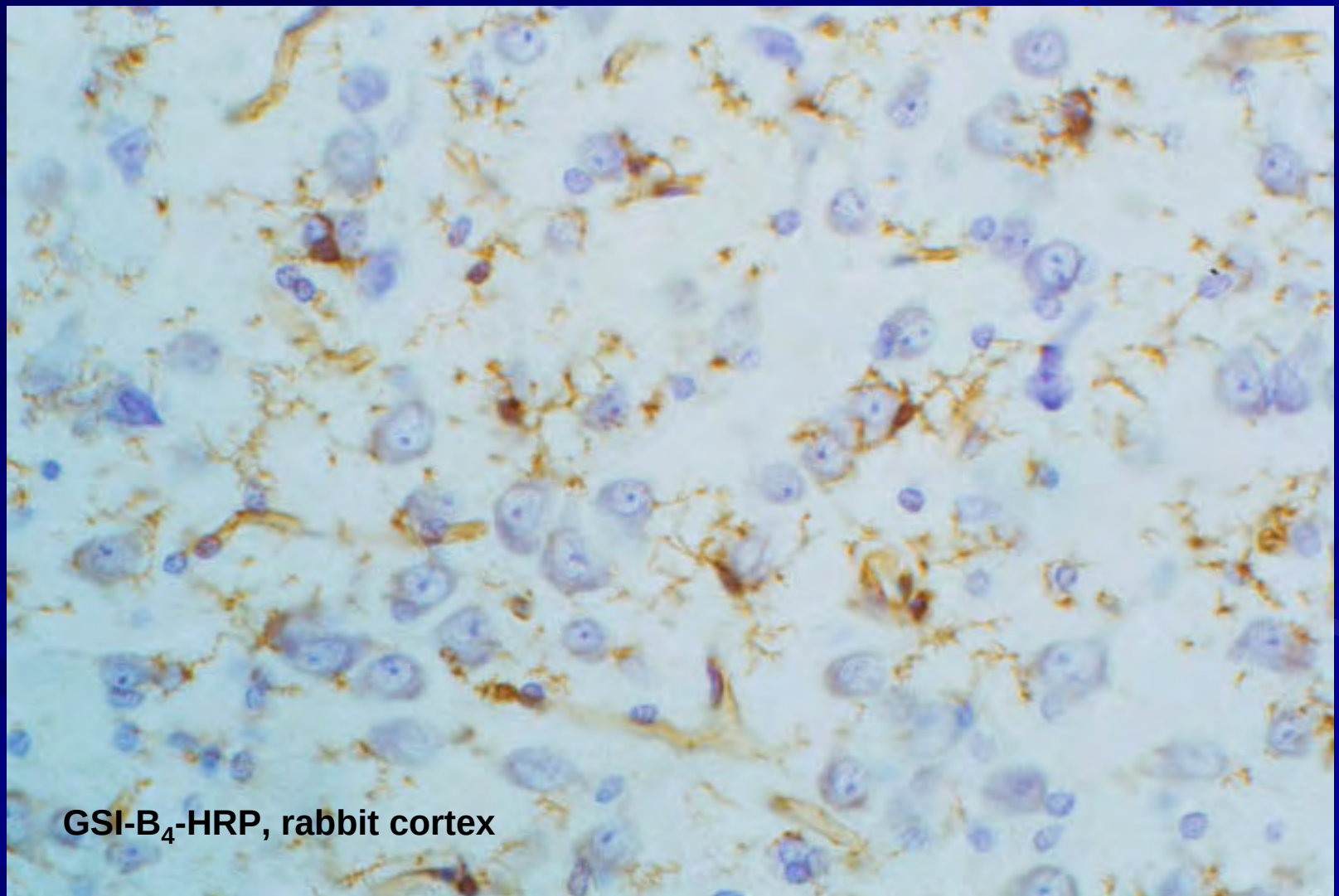
by Wolfgang J. Streit and Carol A. Kincaid-Colton



Salient facts about microglia

- Ubiquitous
- Numerous
- Plasticity
- Immune competence
- Self renewal

Microglia constitute a specialized population of immune competent effector cells in the normal brain.



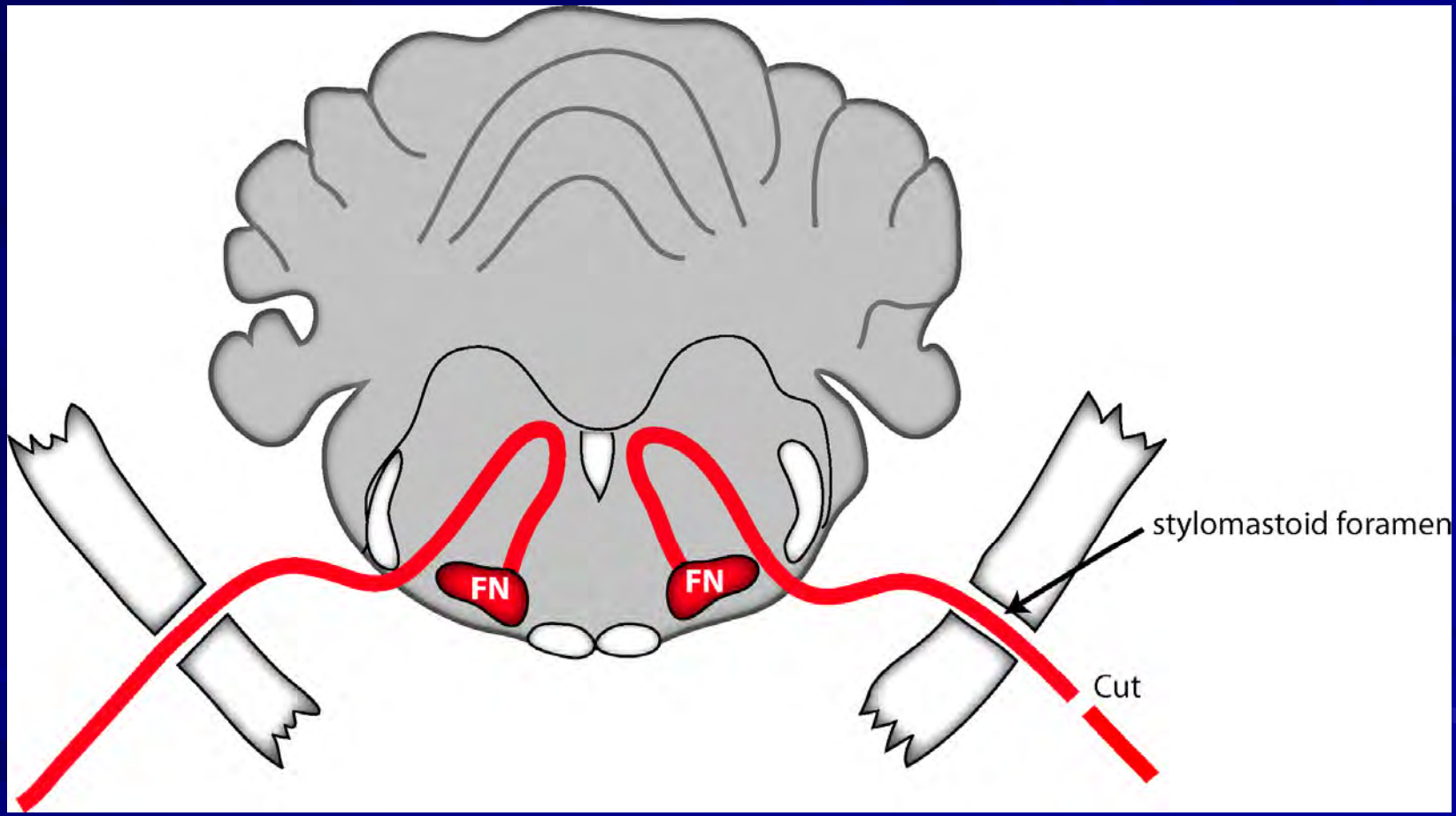
What is microglial activation?

- It is part of the reactive gliosis that occurs invariably during CNS pathology.
- It affects:

1) cell shape
2) cell number (mitosis)
3) phenotype (pattern of surface antigen expression)
4) secretory activity (production of cytokines and growth factors).

- It is likely triggered by signals coming from neurons (neuron-microglia interactions).

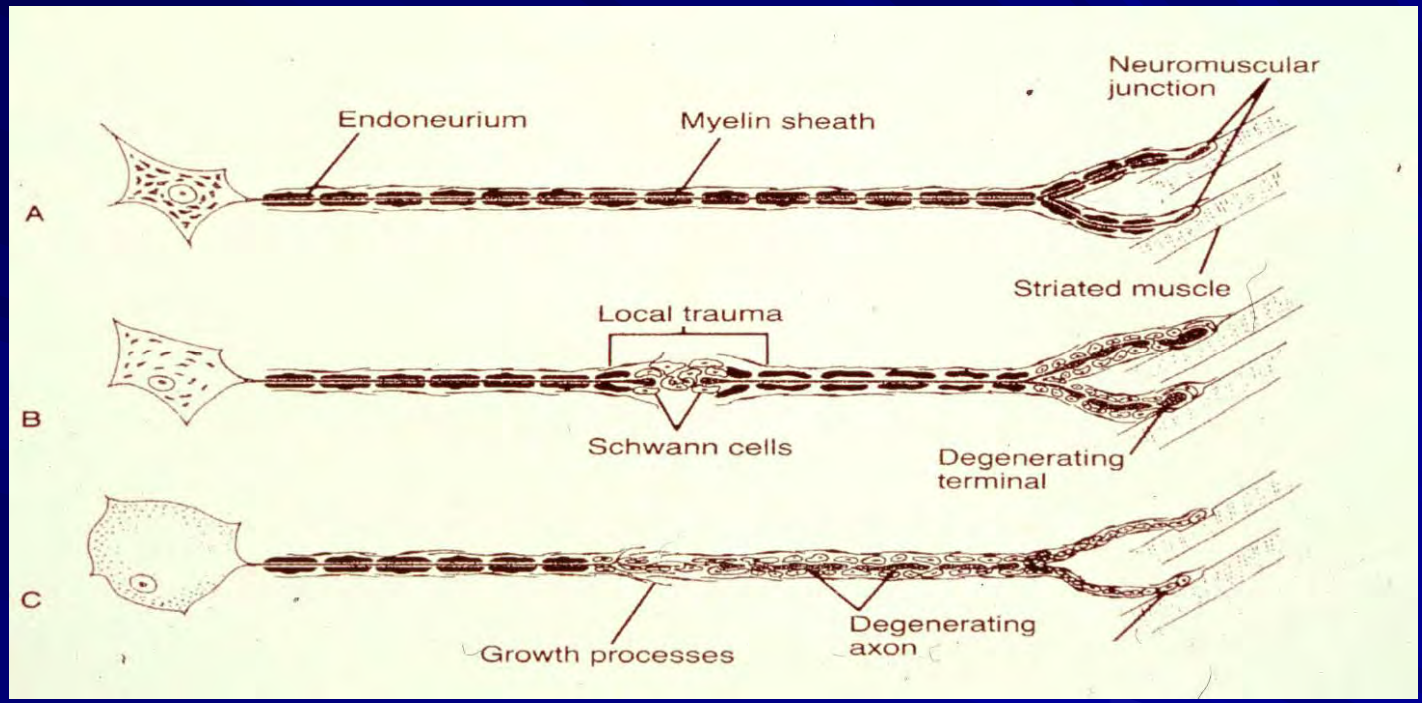
The facial nerve axotomy paradigm



Characteristics of facial nerve model

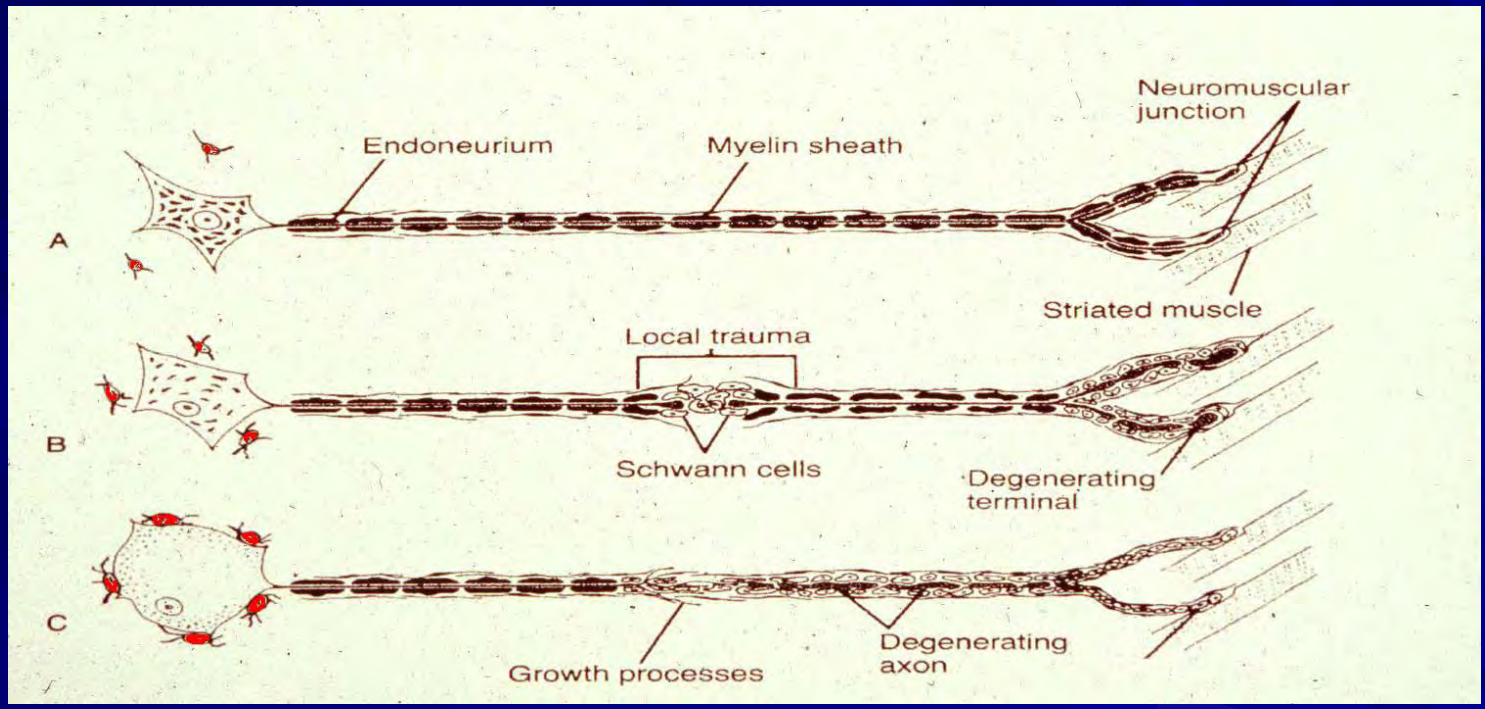
- Reproducibility
- Remote lesion
- Intact blood brain barrier
- Endogenous glial response
- Neuronal regeneration
- Neuron-glia interactions

Mechanisms of PNS Regeneration



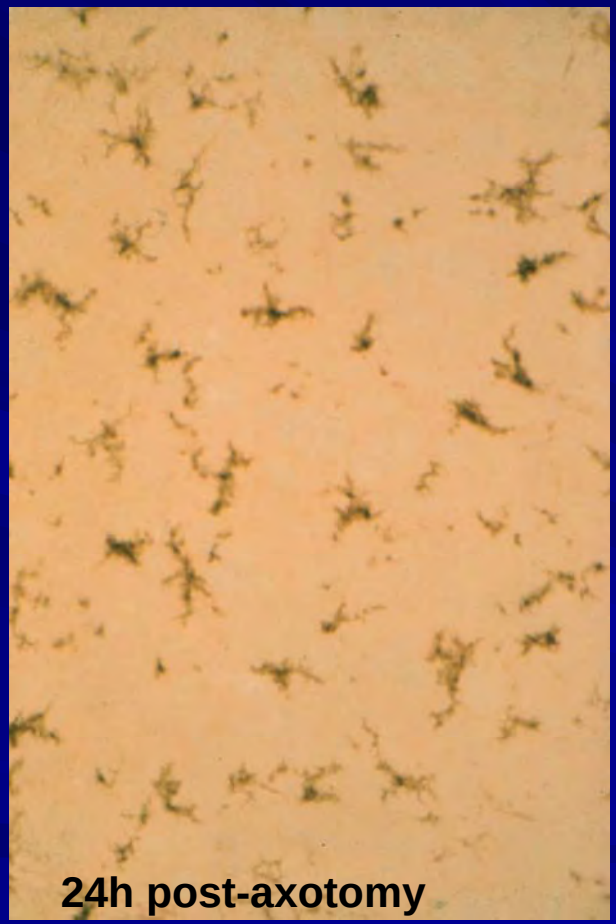
- **PNS neurons**
  - ability to express pro-regenerative genes
- **PNS glia**
  - Schwann cells provide guidance, ECM molecules, trophic factors
  - Macrophages phagocytose debris

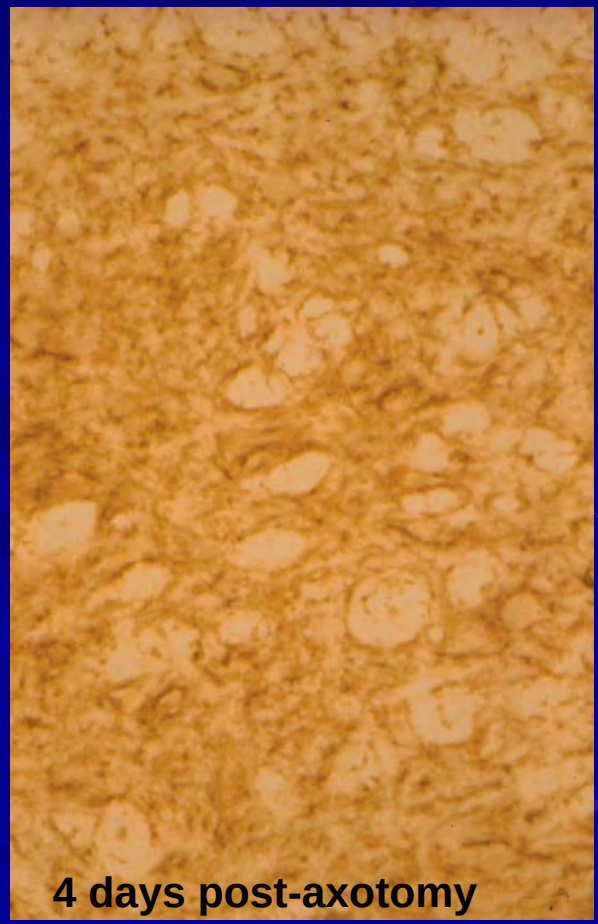
Mechanisms of PNS Regeneration



- **PNS neurons**
  - ability to express pro-regenerative genes
- **CNS glia**
  - are activated following neuronal injury

OX-42 immunohistochemistry reveals hypertrophy and hyperplasia of microglial cells after axotomy



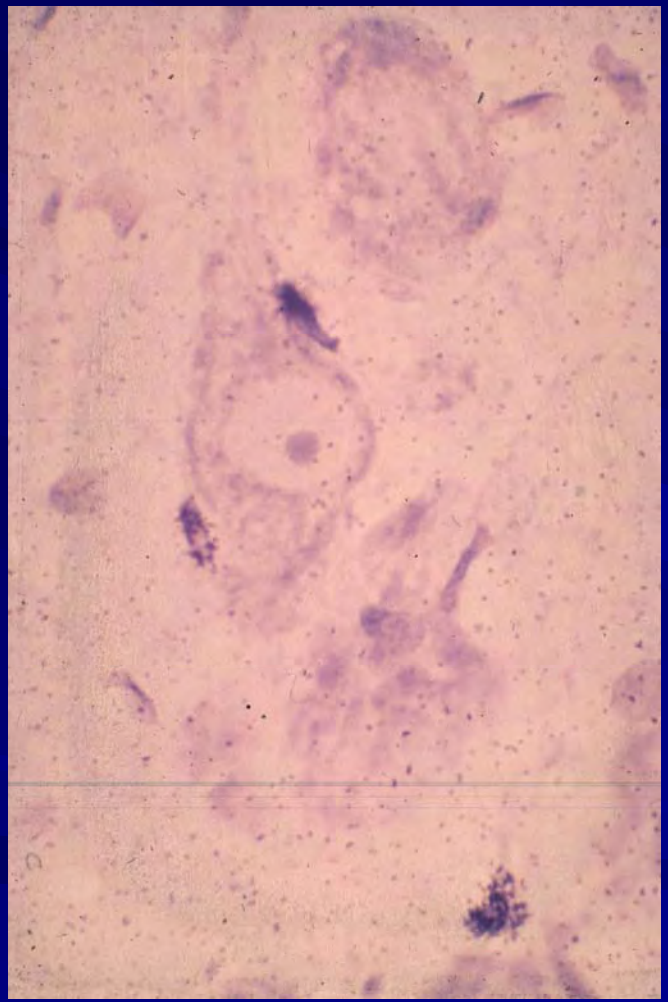


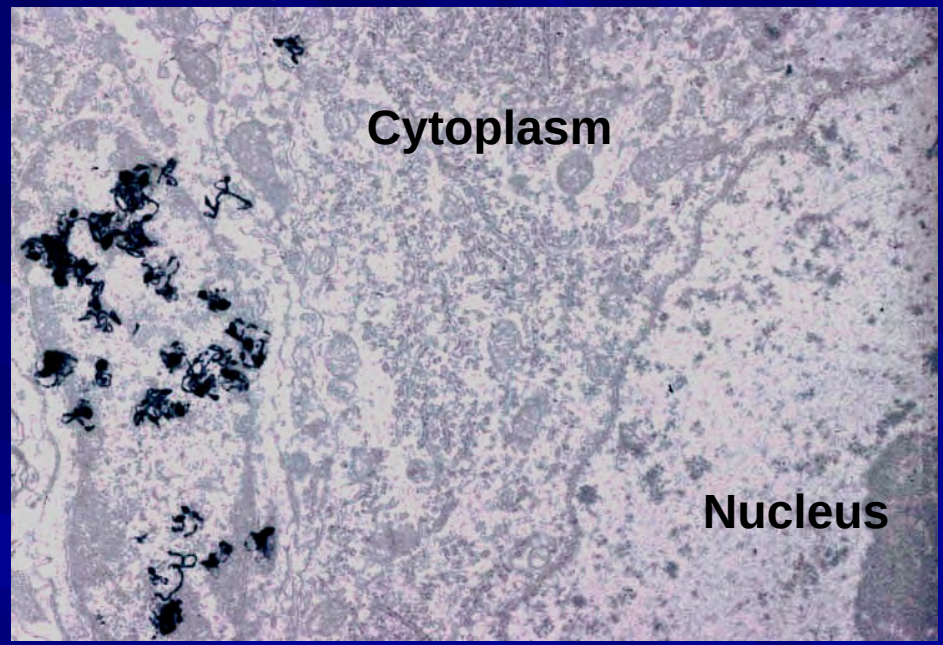
Microglial Immunophenotype

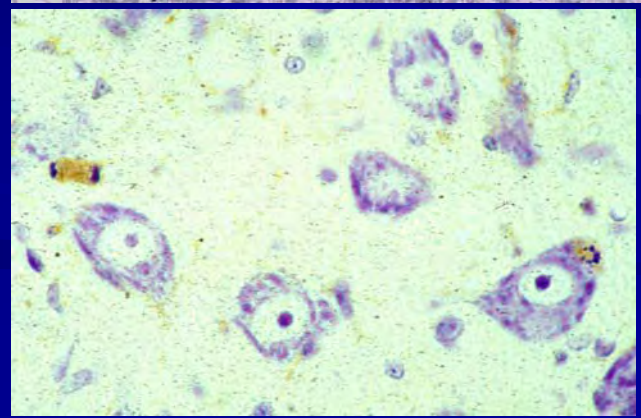
- Complement Receptors
- Fc Receptors
- Immunoglobulins
- Major Histocompatibility Complex (MHC) Antigens Class I and Class II
- CD4 Antigen
- Leukocyte Common Antigen (CD45, CD3?)
- Integrins

Mitosis is part of microglial activation

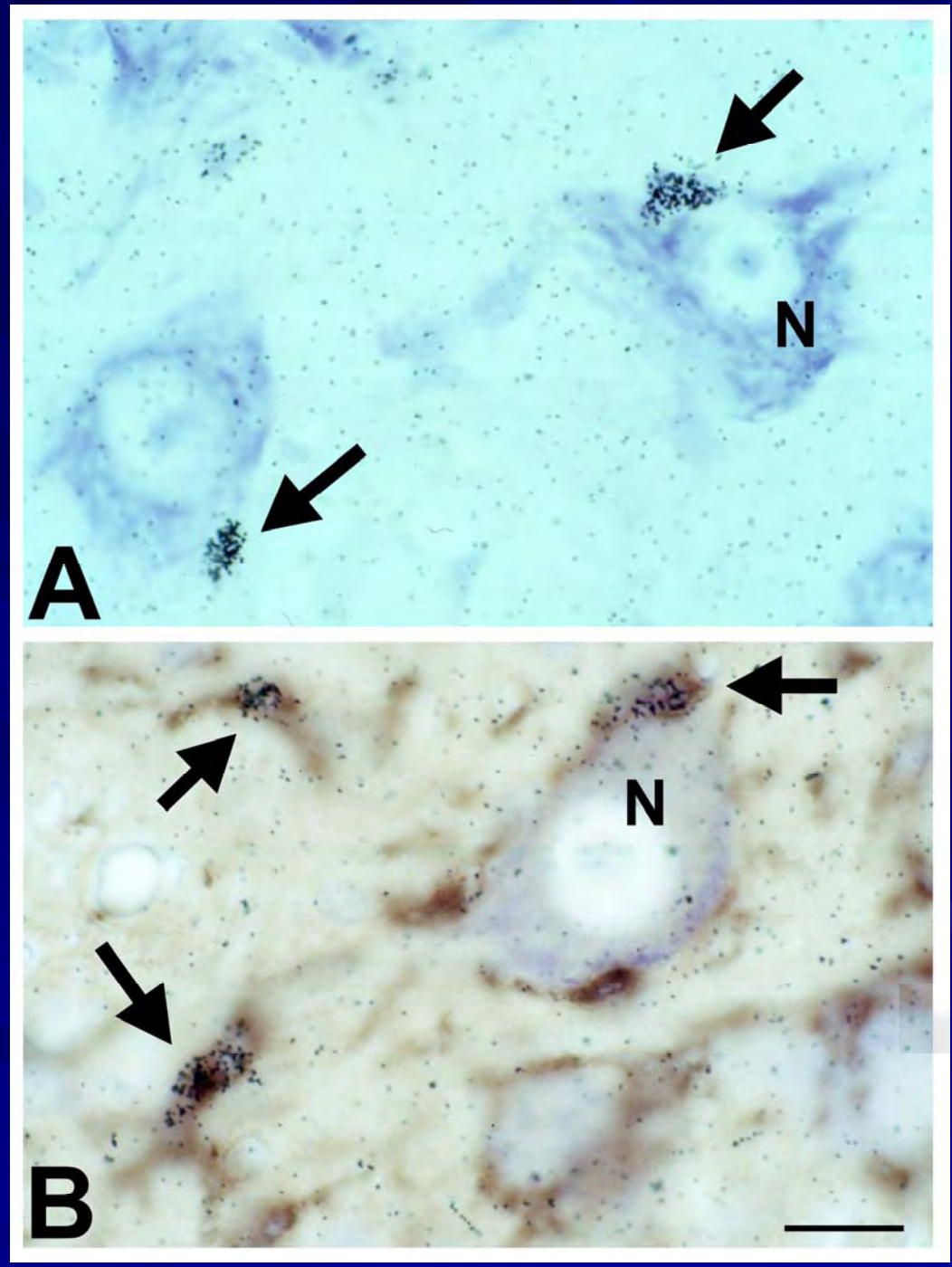
^{3}H-thymidine incorporation autoradiography







Systemic colchicine


A
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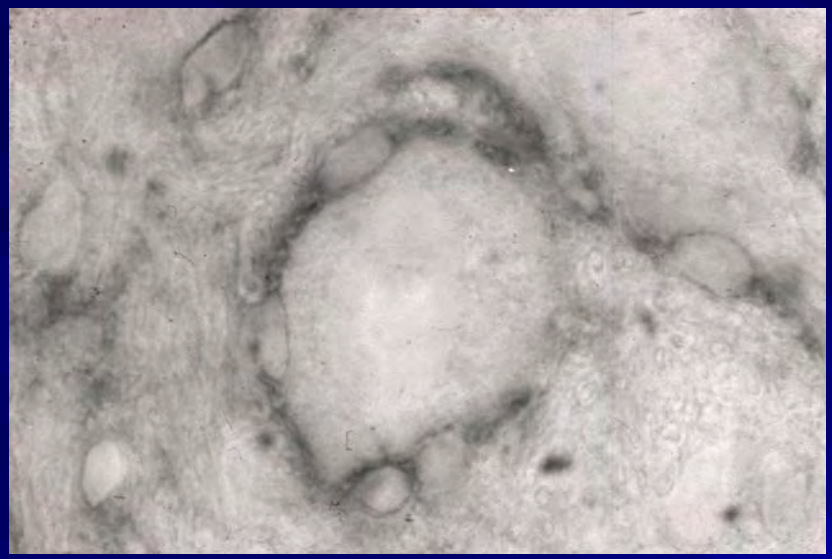
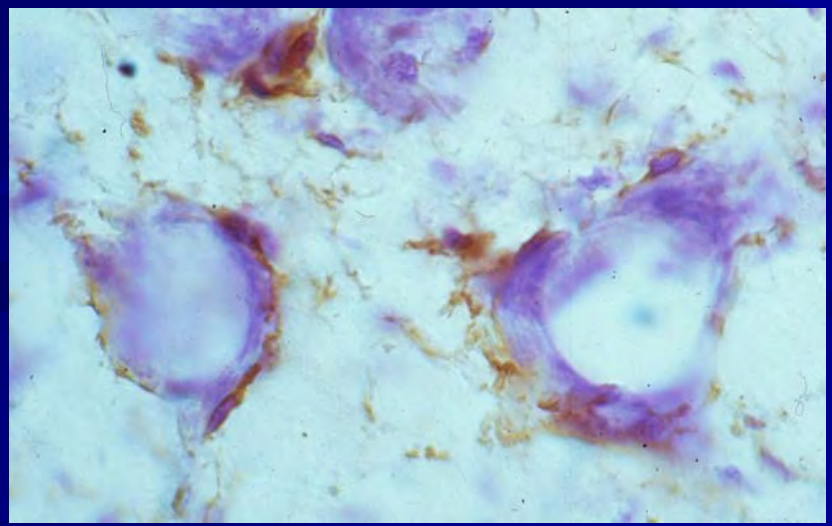
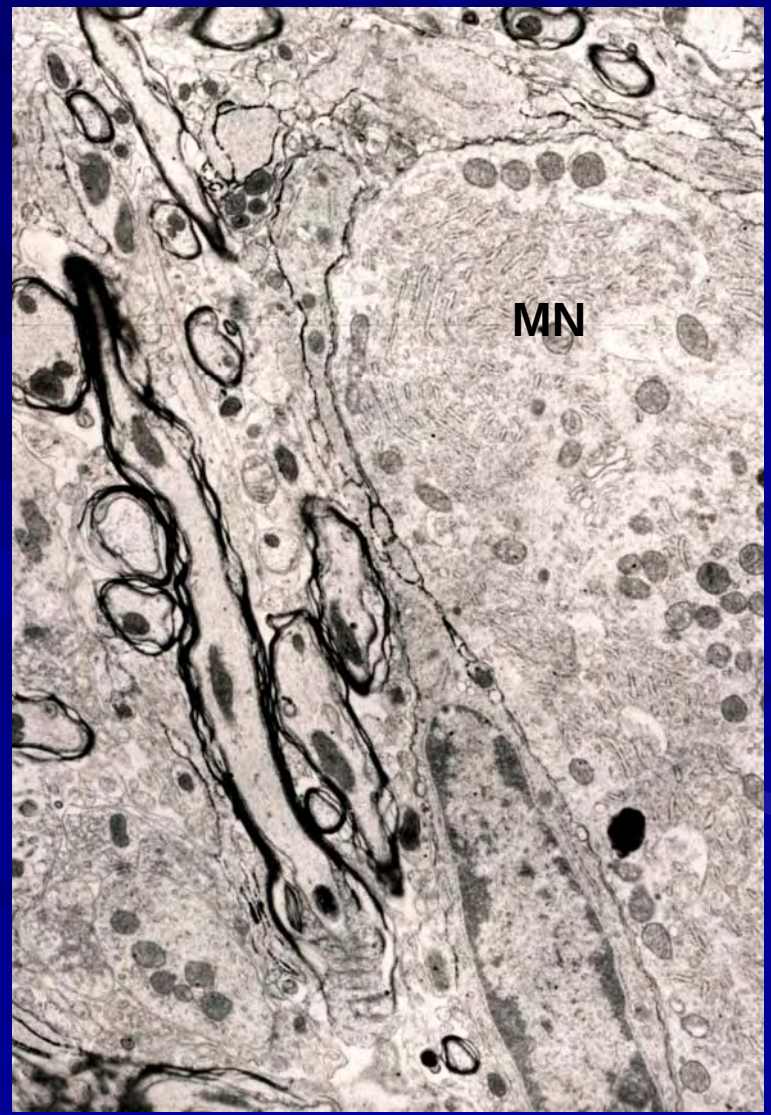
Axotomized motoneurons are ensheathed by perineuronal microglia

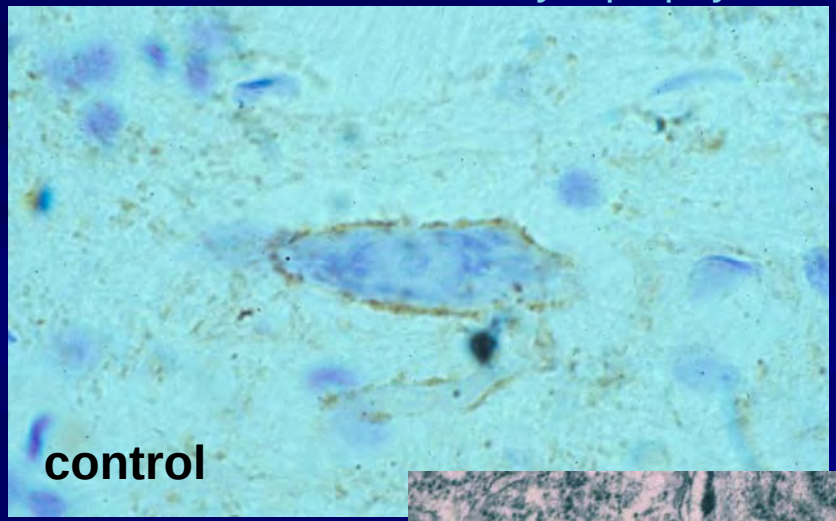


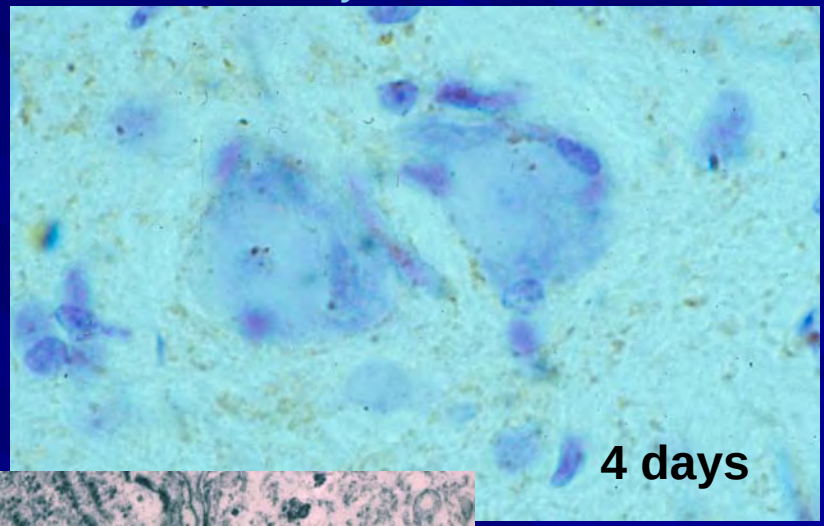




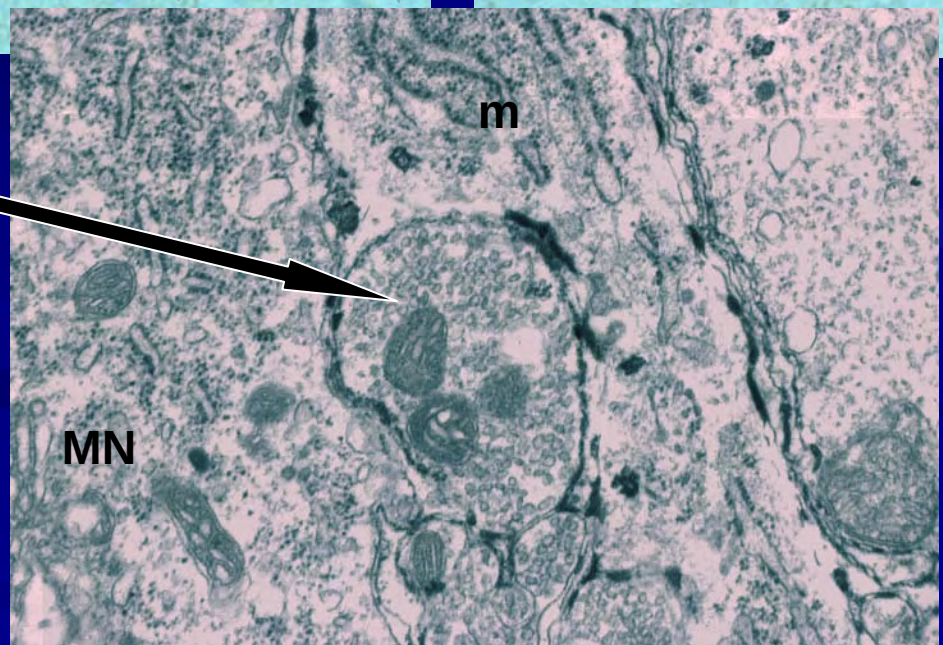
Perineuronal, activated microglia displace synaptic contacts on axotomized motoneurons

Synaptophysin immunohistochemistry

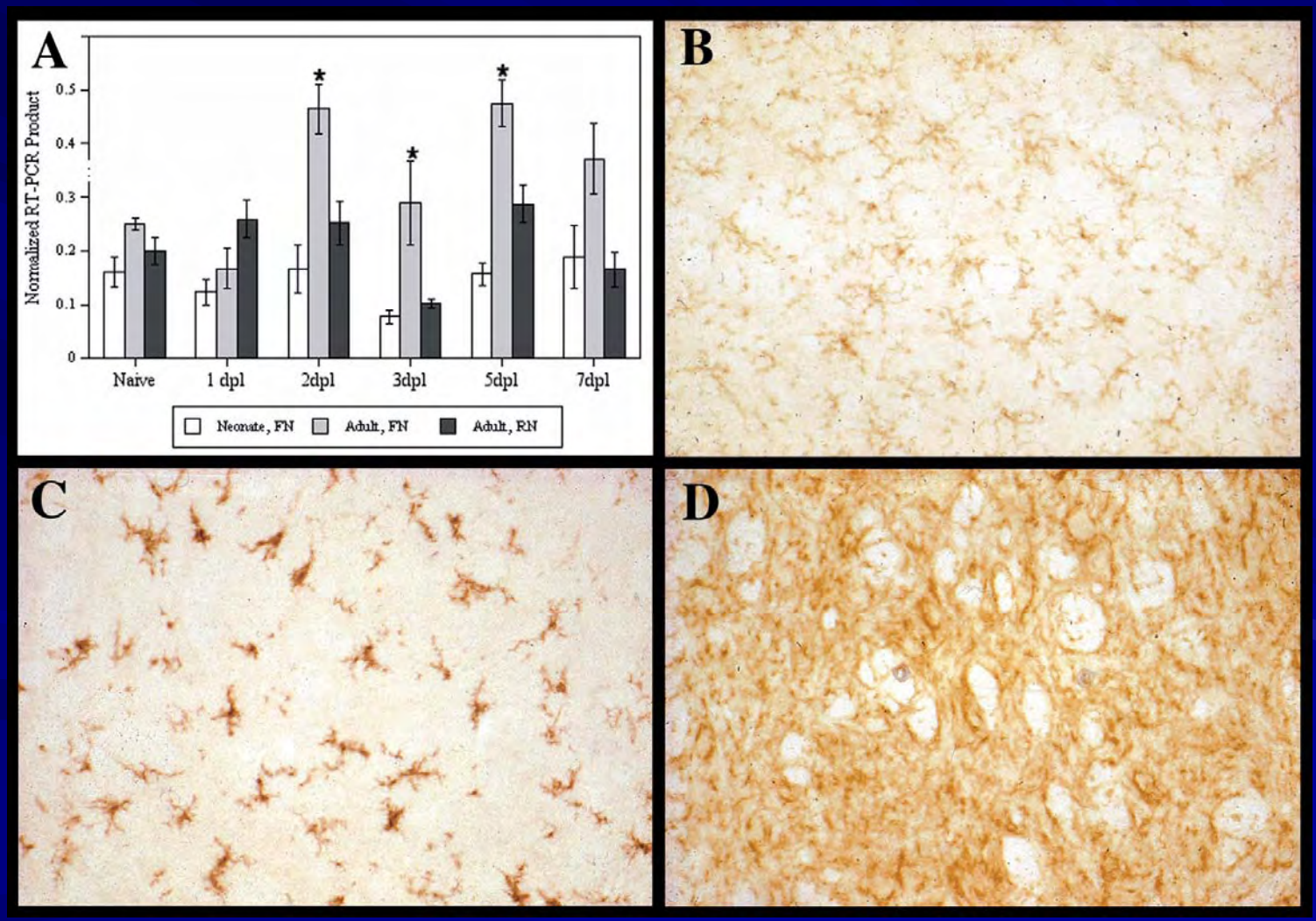




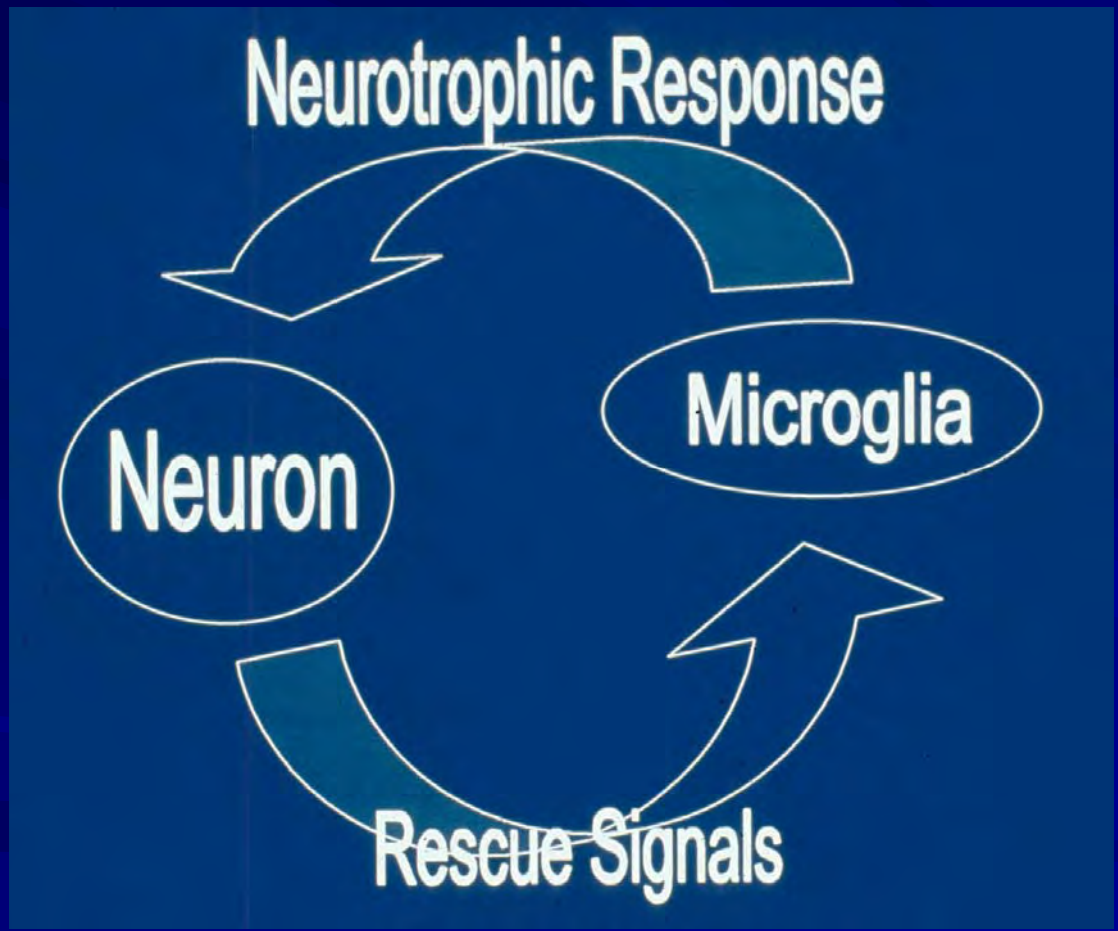
Axon terminal



Progressive microglial activation post-axotomy is accompanied by increased expression of TGF-β1 mRNA

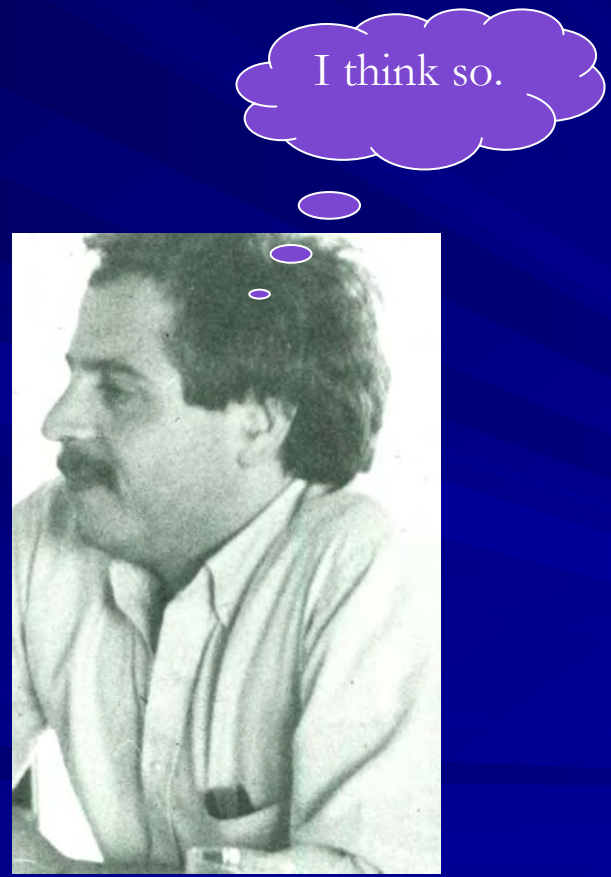


The microglial response to motoneuron axotomy is pro-regenerative, because…..



1) Motoneurons regrow severed axons and reinnervate muscles resulting in restoration of function.
2) Microglial reaction subsides once target reinnervation occurs.
3) Microglia produce TGF-β1, a growth factor for motoneurons.
4) Microglia protect injured neurons from excitatory synaptic input.

So, are activated microglia are our neurons' friends?



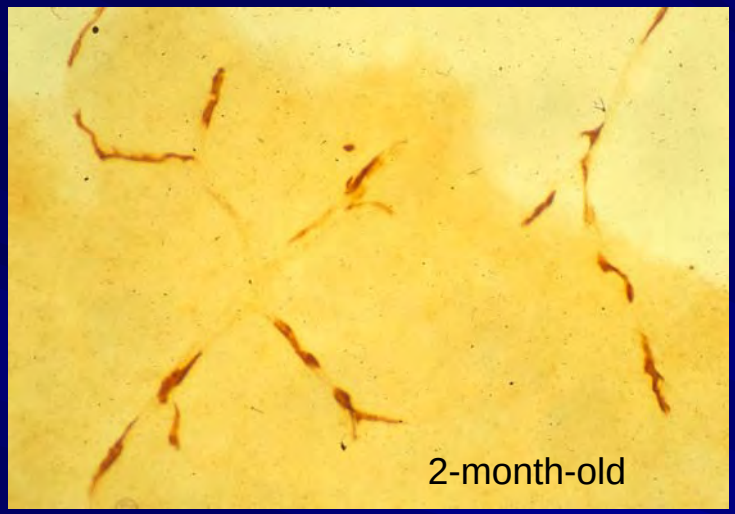
Reactive gliosis, neuroinflammation, and CNS autoimmune disease

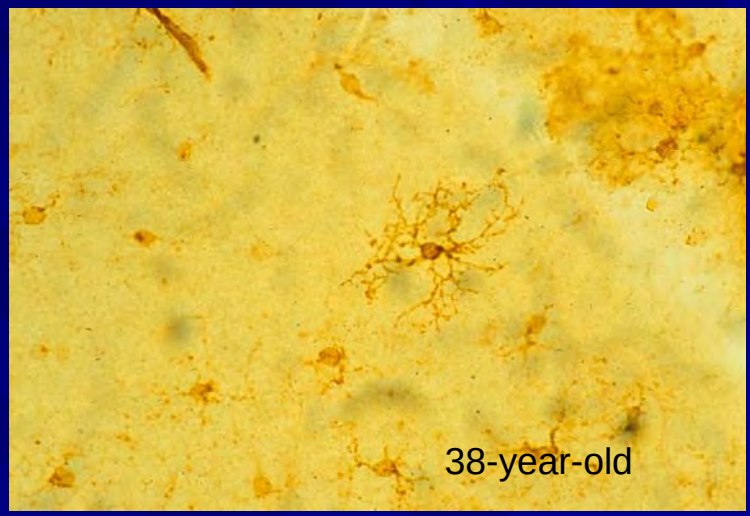
- Reactive gliosis: occurs during all pathological insults to the CNS; triggered by altered neuronal signaling.
- Neuroinflammation: popular, but non-specific term currently in use to include reactive gliosis. Not used to discriminate between “response to injury” and “autoimmune attack”.
- CNS autoimmune disease: occurs to a very limited extent. The only good example is multiple sclerosis.

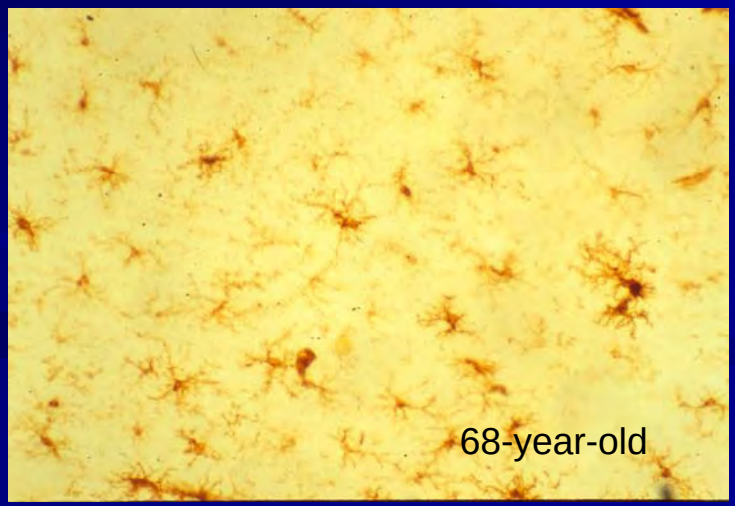
Microglia and Aging

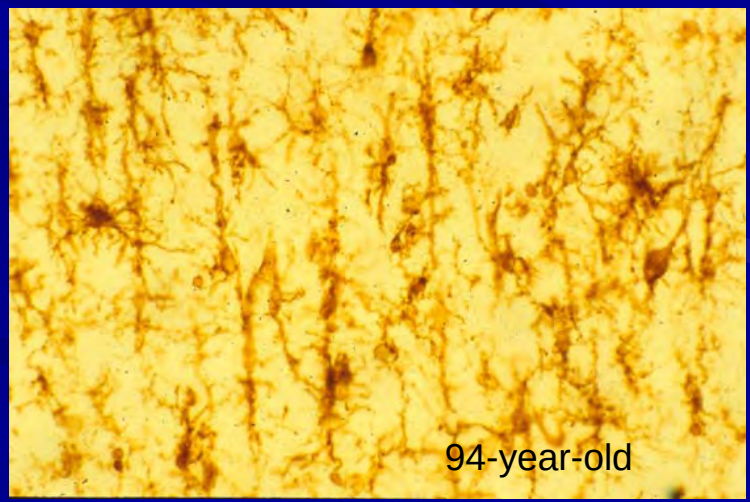
Current view: There is microglial activation (neuroinflammation) with aging.

There is an increase of HLA-DR (MHC II)-positive microglia in the brain with aging.

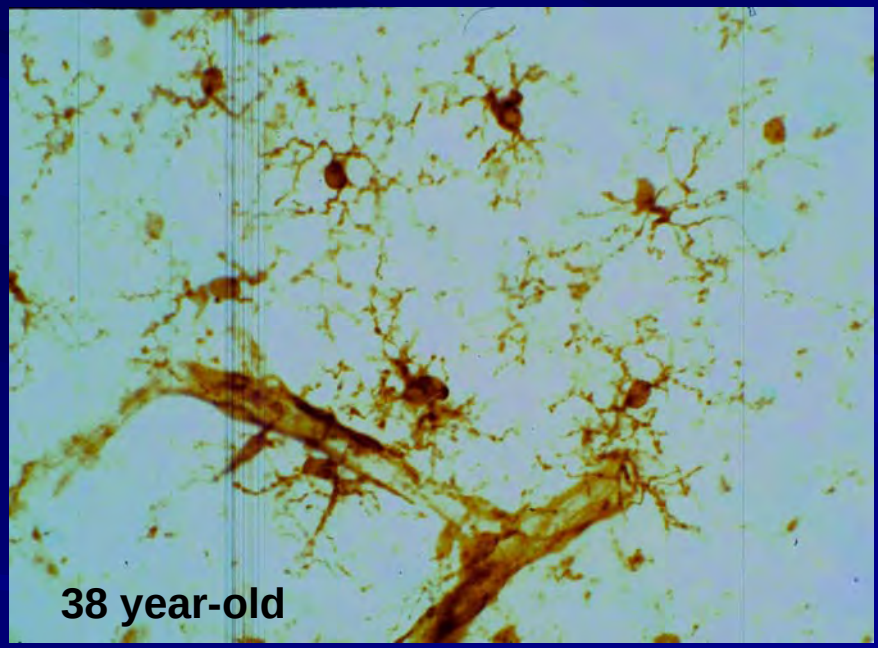


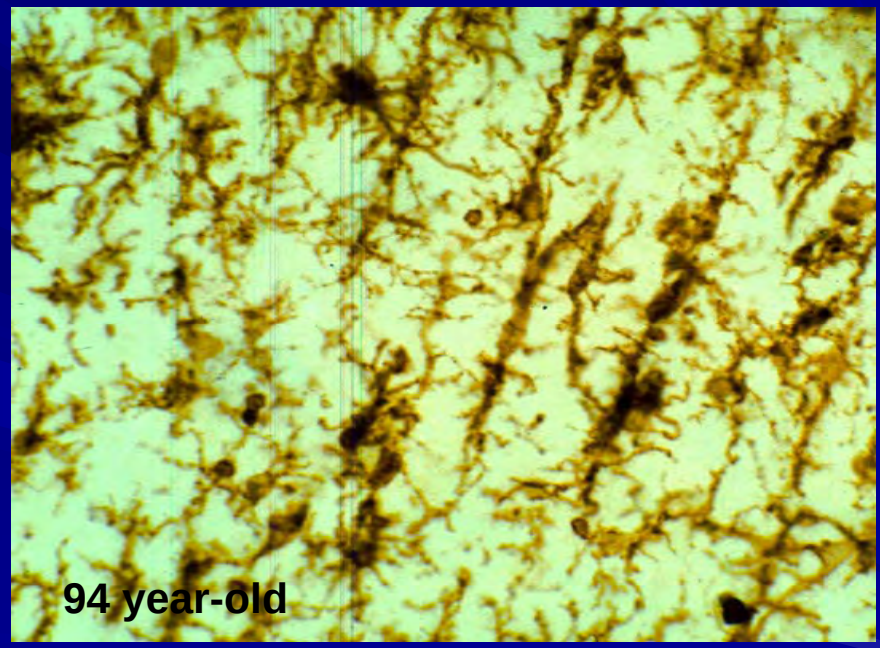






Microglial morphology and phenotype change with aging.





Progressive microglial activation with aging is unexplained.

Microglia and Aging in the Brain

CALEB E. FINCH, TODD E. MORGAN, IRINA ROZOVSKY, ZHONG XIE, RICHARD WEINDRUCH, AND TOMAS PROLLA

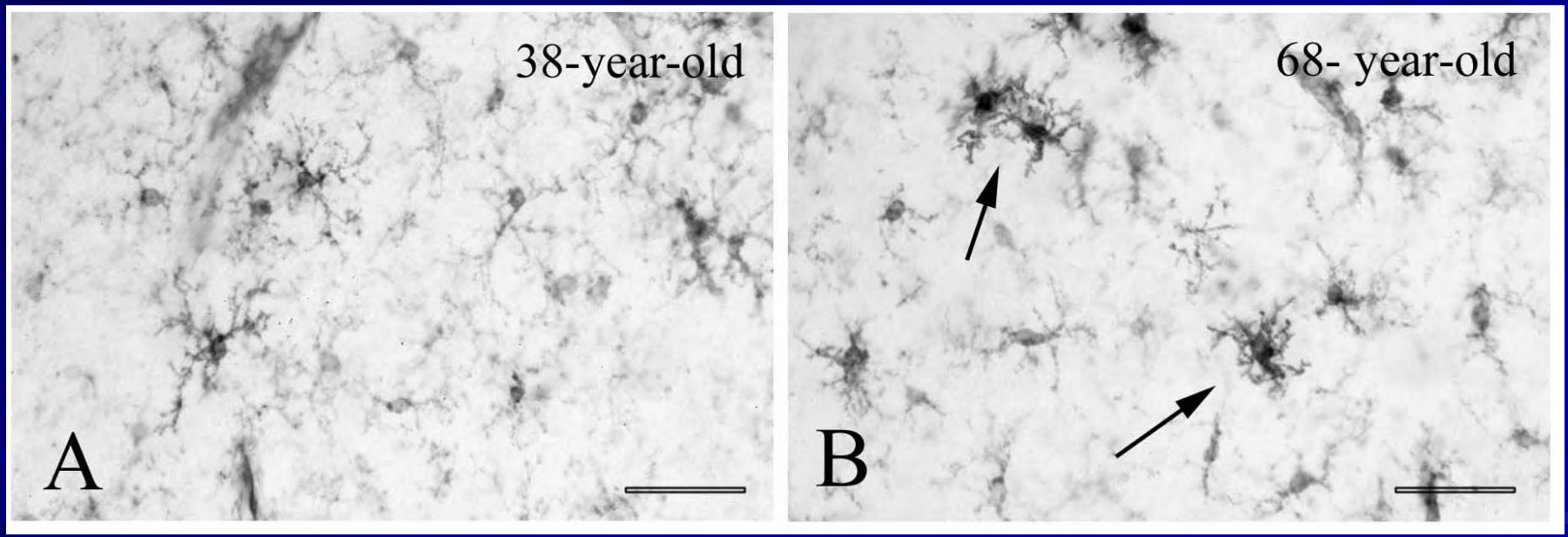
1. Introduction

Microglial activation during normal aging in the CNS is puzzling. In general, microglial activation is associated with neuron death, blood-brain barrier disruption, or invading lymphocytes, whereas, as we shall discuss, there is little evidence for such changes during normal aging. Microglial age changes may also interact with Alzheimer's disease, which increases markedly during aging, as well as with inflammatory processes of aging in peripheral tissues.

In: Microglia *in the regenerating and degenerating central nervous system.* Streit, W.J. (ed.), *Springer Verlag, New York, 2002, pp 275-305.*

Is there really progressive microglial activation with aging?

The aging brain contains deformed (dystrophic) microglia.



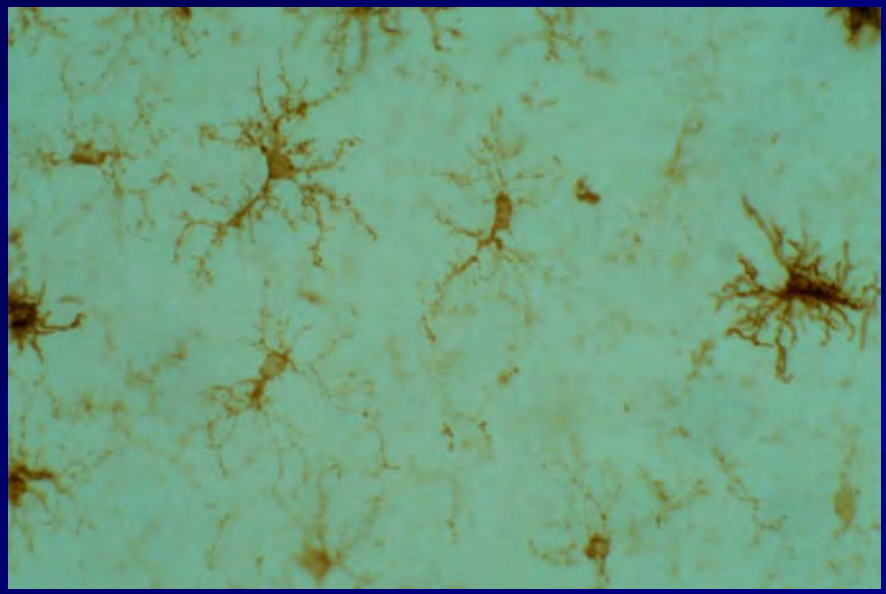
Incidence of dystrophic microglia increases with age.

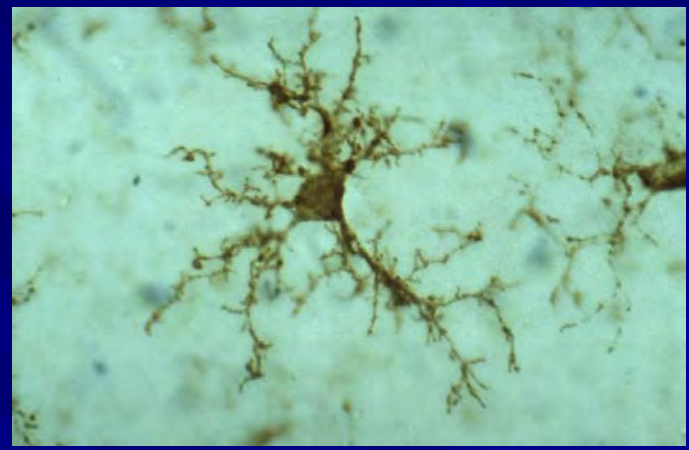
Table 1. Patient data and quantification of dystrophic microglia.

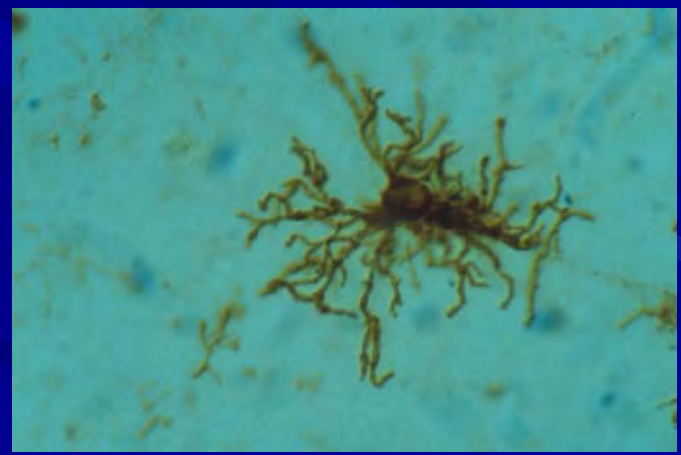
Age	Sex	Race	PMI	Cause of Death	Instances of Dystrophic Microglia
38	Male	White	< 7 h	Acute Cardiac Dysrhythmia	9
68	Male	White	6 h	Neck Fracture due to MVA	83

PMI: Post-mortem Interval MVA: Motor Vehicle Accident

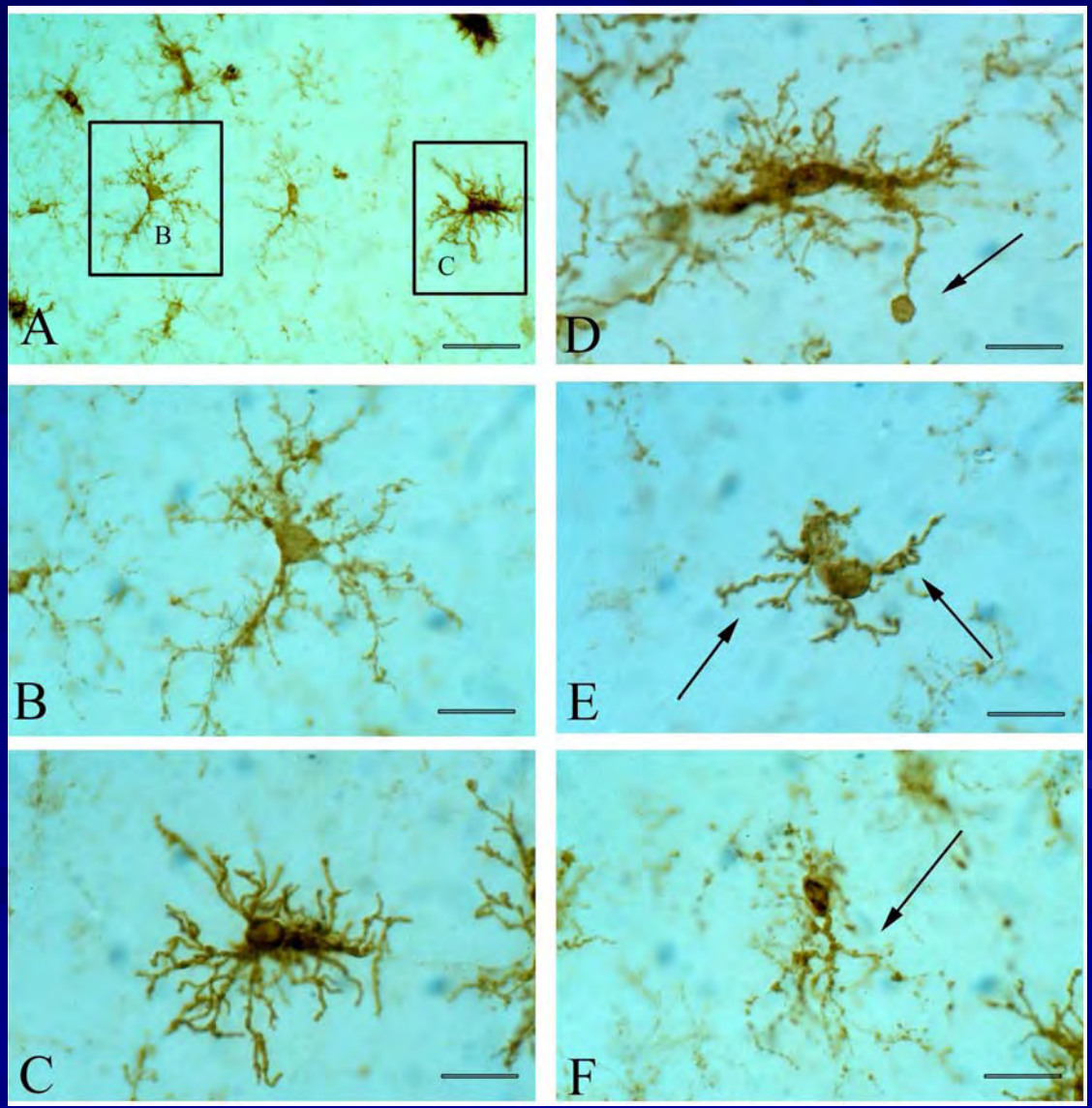
Some dystrophic microglia are deramified.



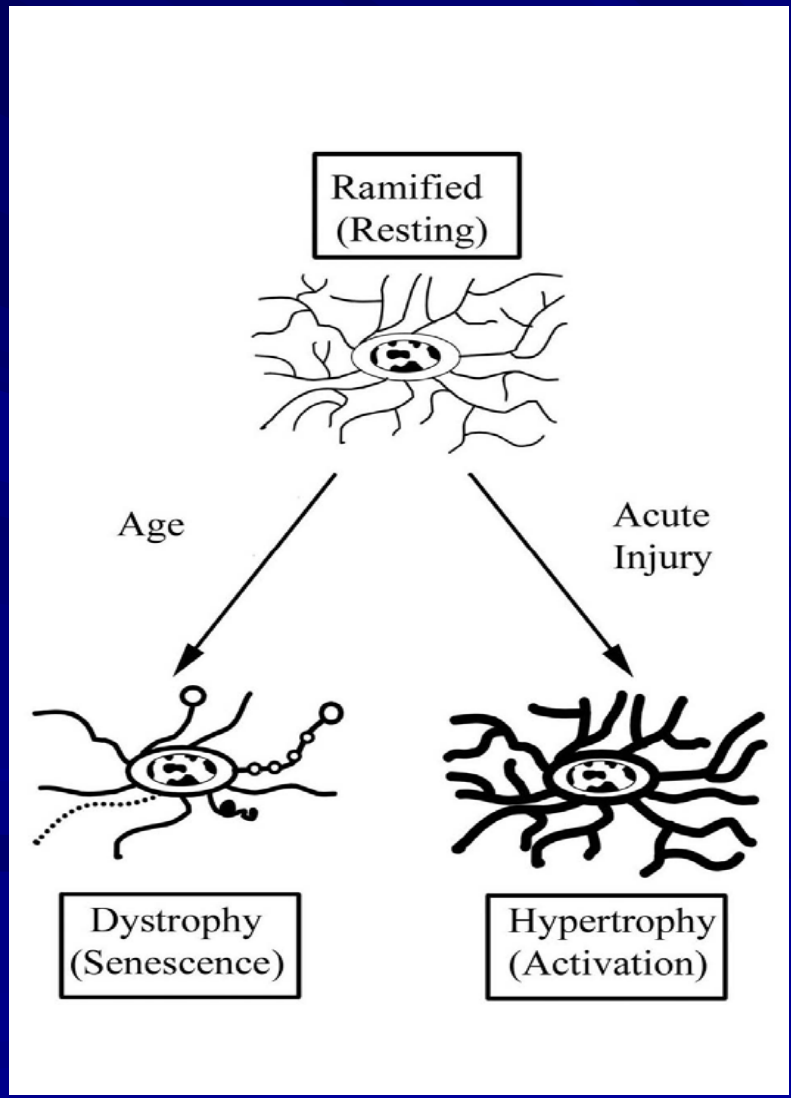




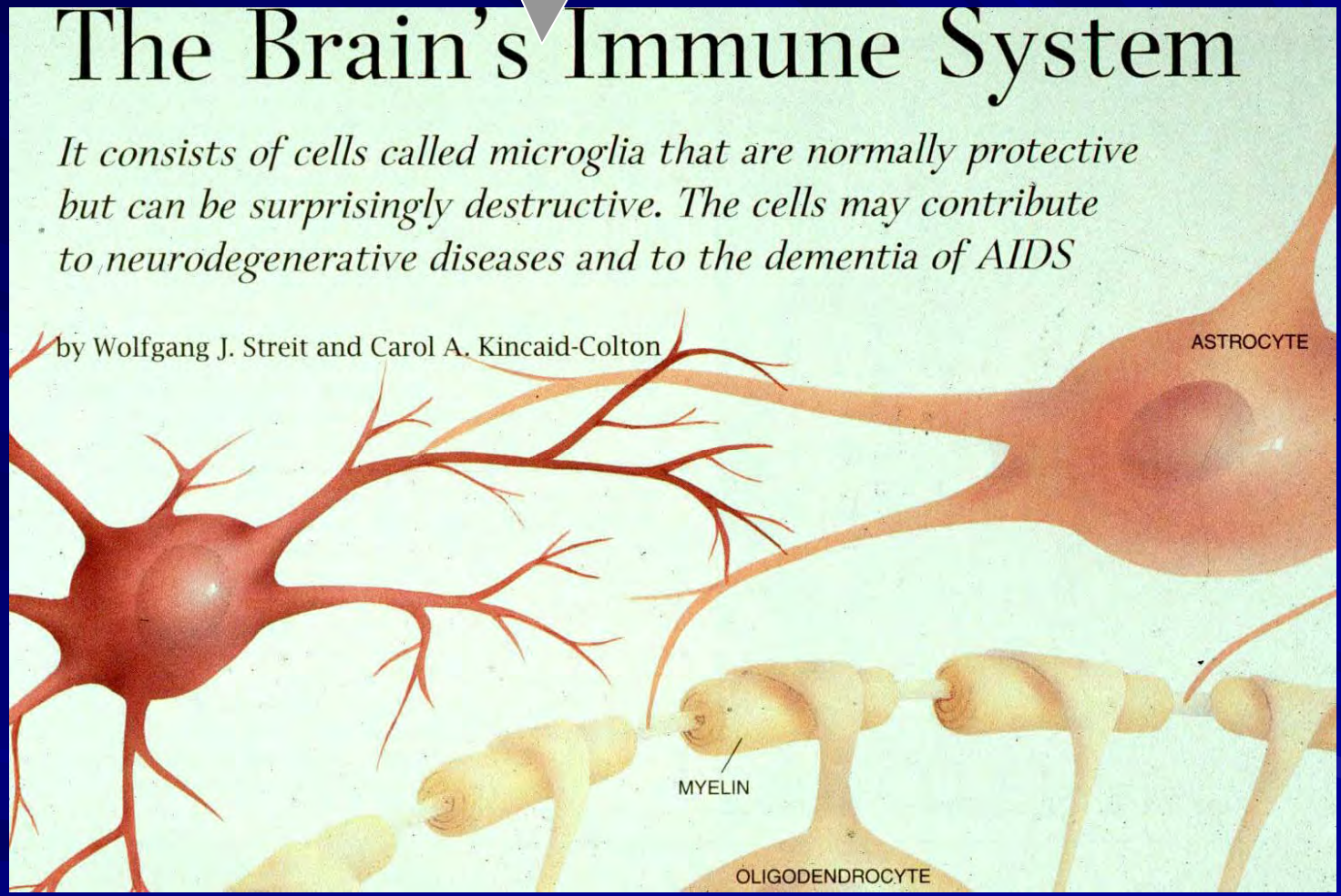
Other structural abnormalities that occur in microglia with aging include spheroids and cytoplasmic fragmentations.



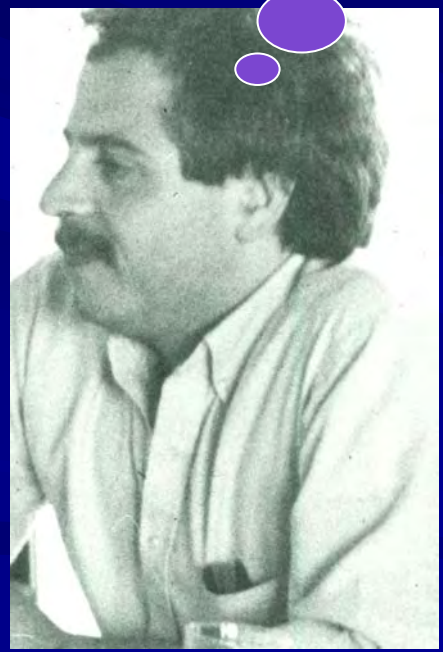
With aging there is microglial dystrophy, not hypertrophy.



Aging


The Brain's Immune System
It consists of cells called microglia that are normally protective but can be surprisingly destructive. The cells may contribute to neurodegenerative diseases and to the dementia of AIDS
by Wolfgang J. Streit and Carol A. Kincaid-Colton
ASTROCYTE
MYELIN
OLIGODENDROCYTE

Are microglia undergoing cell senescence?



Why is it important to study how the brain's immune system ages?

- There is reason to think that microglia are important for neurons to survive.
- Microglia are the only mature cell type in the brain that can divide. Are they subject to replicative senescence?

Replicative senescence:

All mitotically active, non-cancerous cells can undergo only a limited number of cell divisions before they die.

Ways to look at cell senescence

- Proliferation assays *in vivo and in vitro*

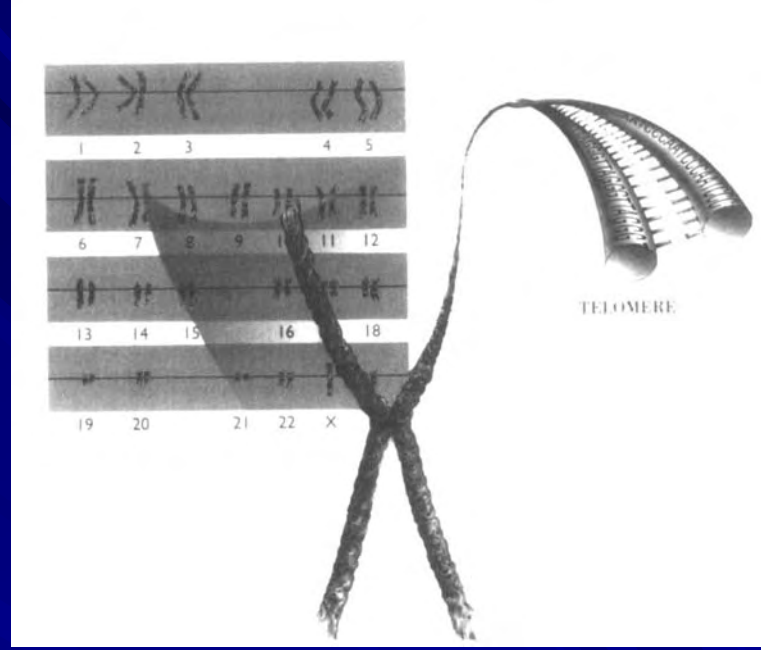
  (microglia divide after neuronal injury)
- Cell morphology

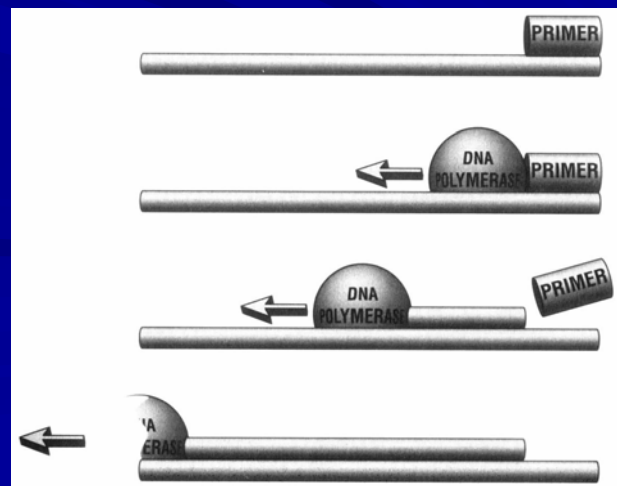
  (microglia become dystrophic in the aged brain)
- Measurement of telomere length and telomerase activity

  (microglial telomeres shorten with time *in vitro)*
- Biochemical and genetic changes

Telomeres

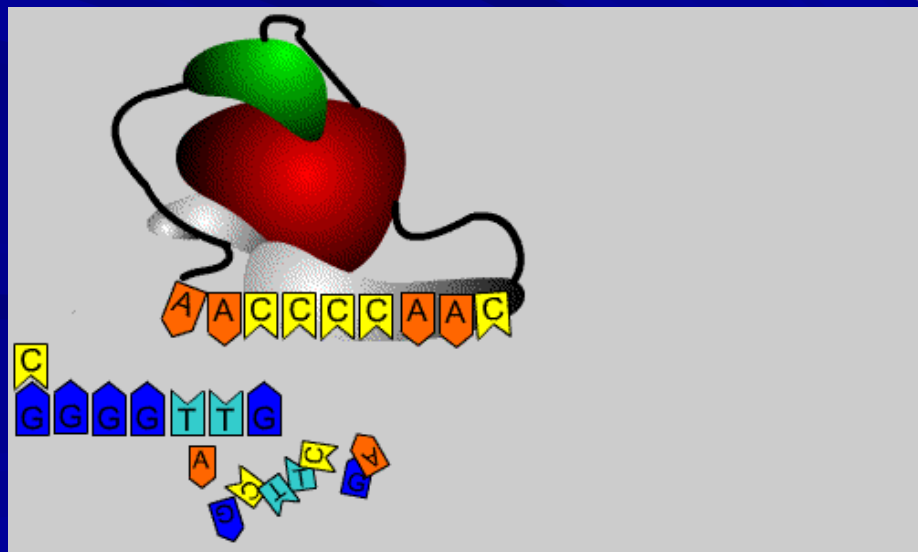
- Specialized structures at physical ends of chromosomes
- Vertebrate telomeres: $(TTAGGG)_n$
- Species-specific length
  - 5 - 20kb (humans)
  - 20 - 150kb (mice)
- Loss of 50 - 200 bp telomeric DNA/cell division
  - Young human fibroblasts (20 - 25 kb)
  - Senescent human fibroblasts (4 - 10 kb)
- Telomeres erode until one telomere reaches critically-short length (Hayflick Limit), then cell enters senescence.



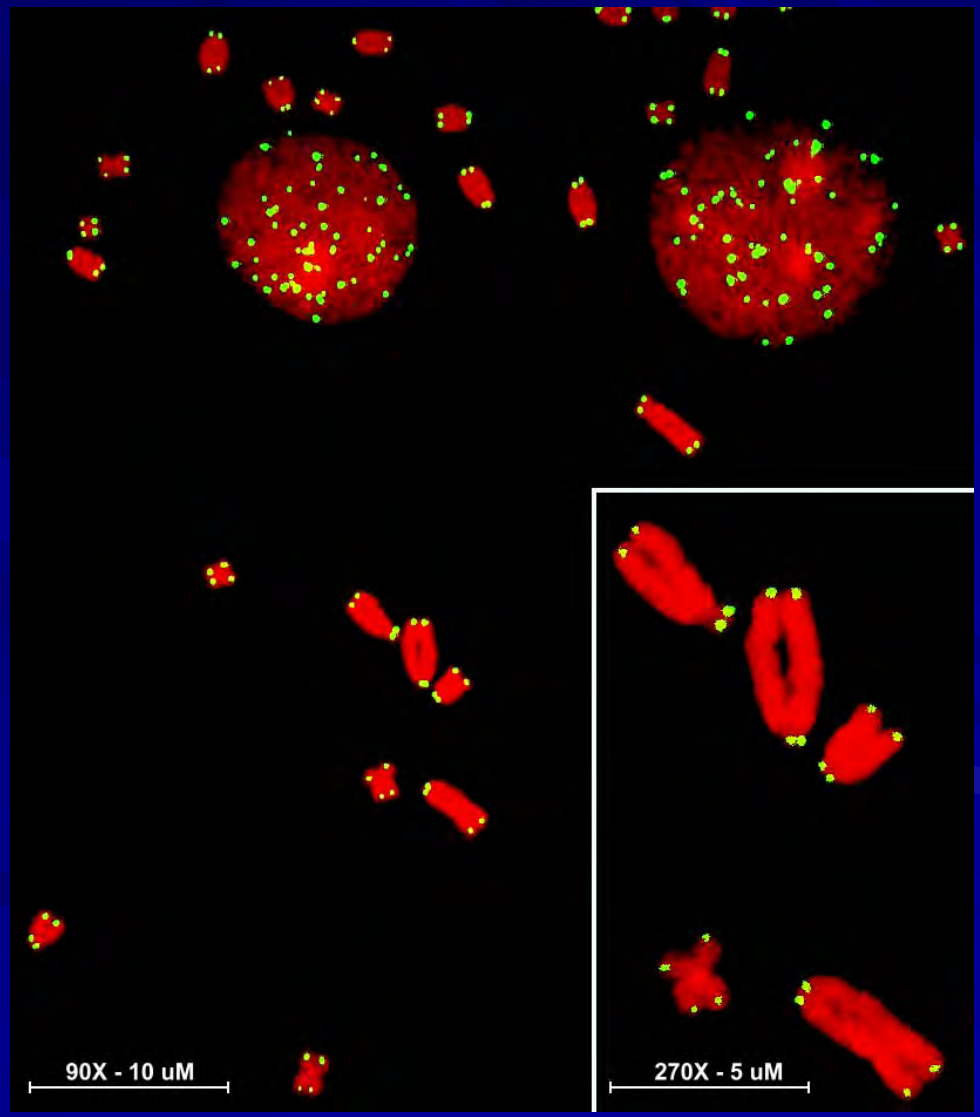


Telomerase

- Ribonucleoprotein enzyme complex
  - RNA component (hTR)
    - Expressed in all cells
  - Protein component (hTRT)
    - Only expressed in gametic cells, stem cells, certain WBC's, and cancer
  - Mediates RNA-dependent synthesis of telomeric DNA
    - Prevents telomere shortening

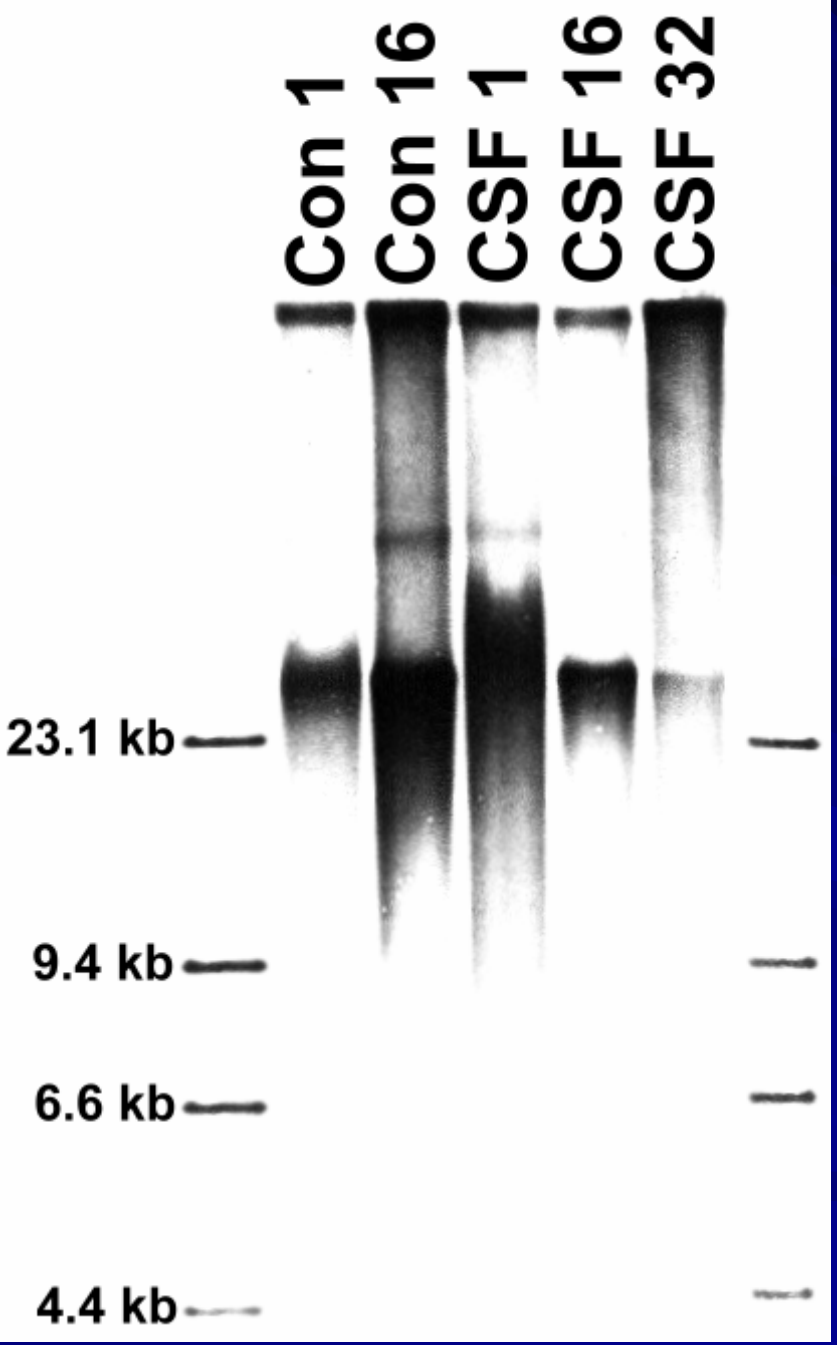


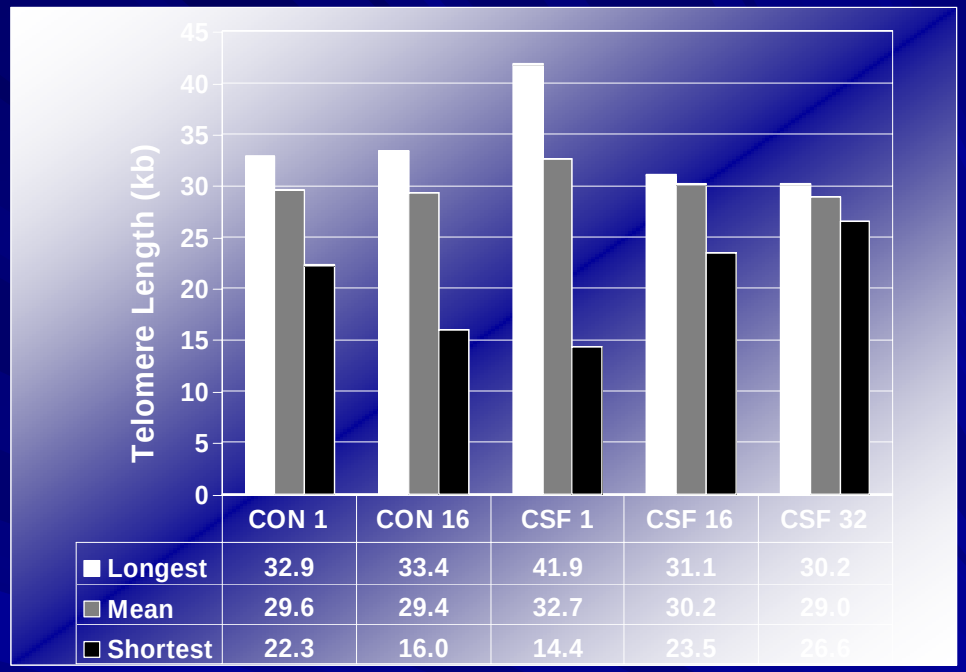
Microglial cells have telomeres.



Flanary and Streit, Glia 45:75-88, 2004

Microglia Telomere Length

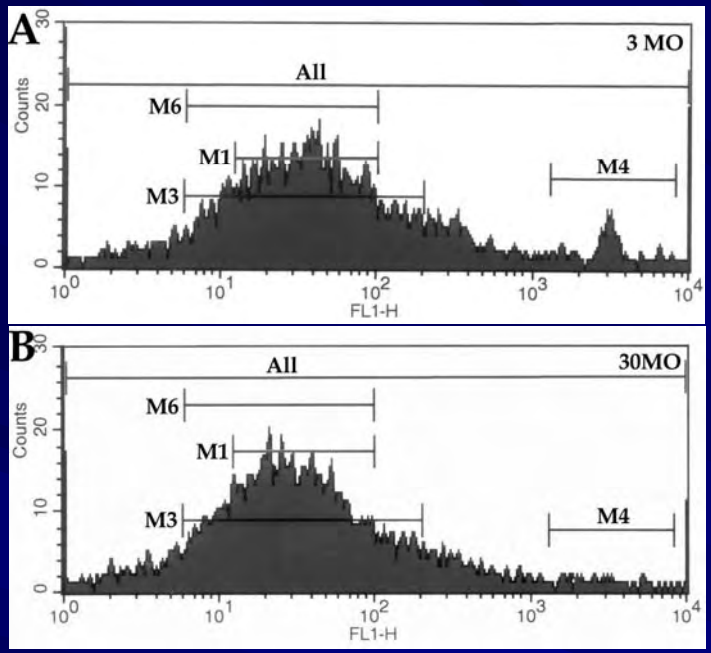




	CON 1	CON 16	CSF 1	CSF 16	CSF 32
Longest	32.9	33.4	41.9	31.1	30.2
Mean	29.6	29.4	32.7	30.2	29.0
Shortest	22.3	16.0	14.4	23.5	26.6

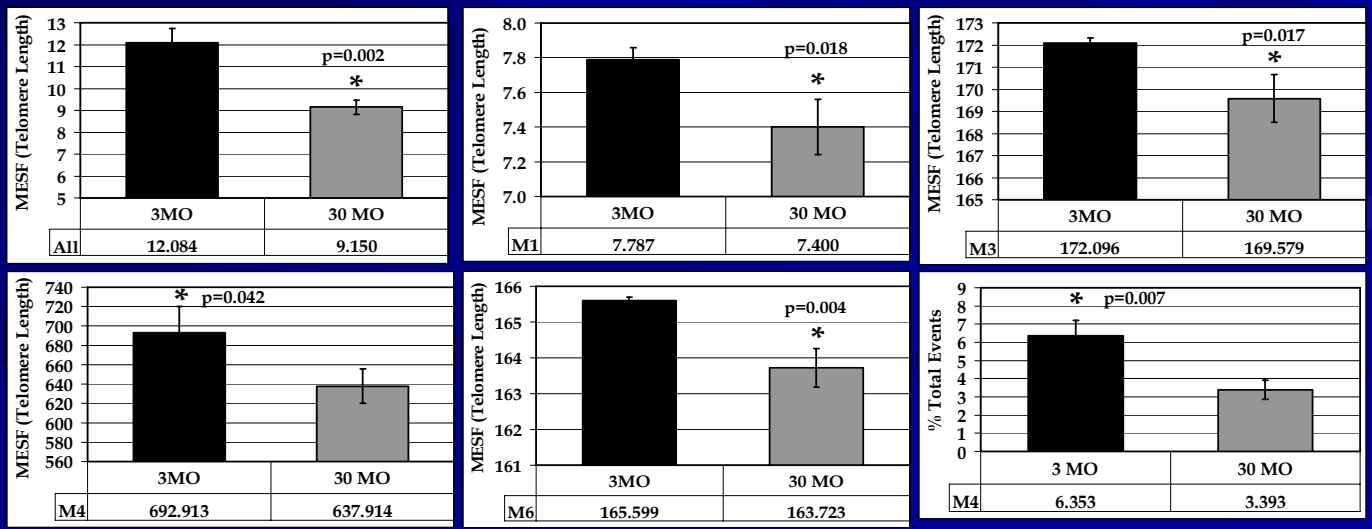
- Control
  - Decreases with time in culture
  - Cells senesce before day 32 *in vitro*
- CSF-treated
  - Longest telomeres shorten
  - Shortest telomeres lengthened
  - Cells senesce ~ d. 32

Does microglial telomere shortening happen *in vivo?*



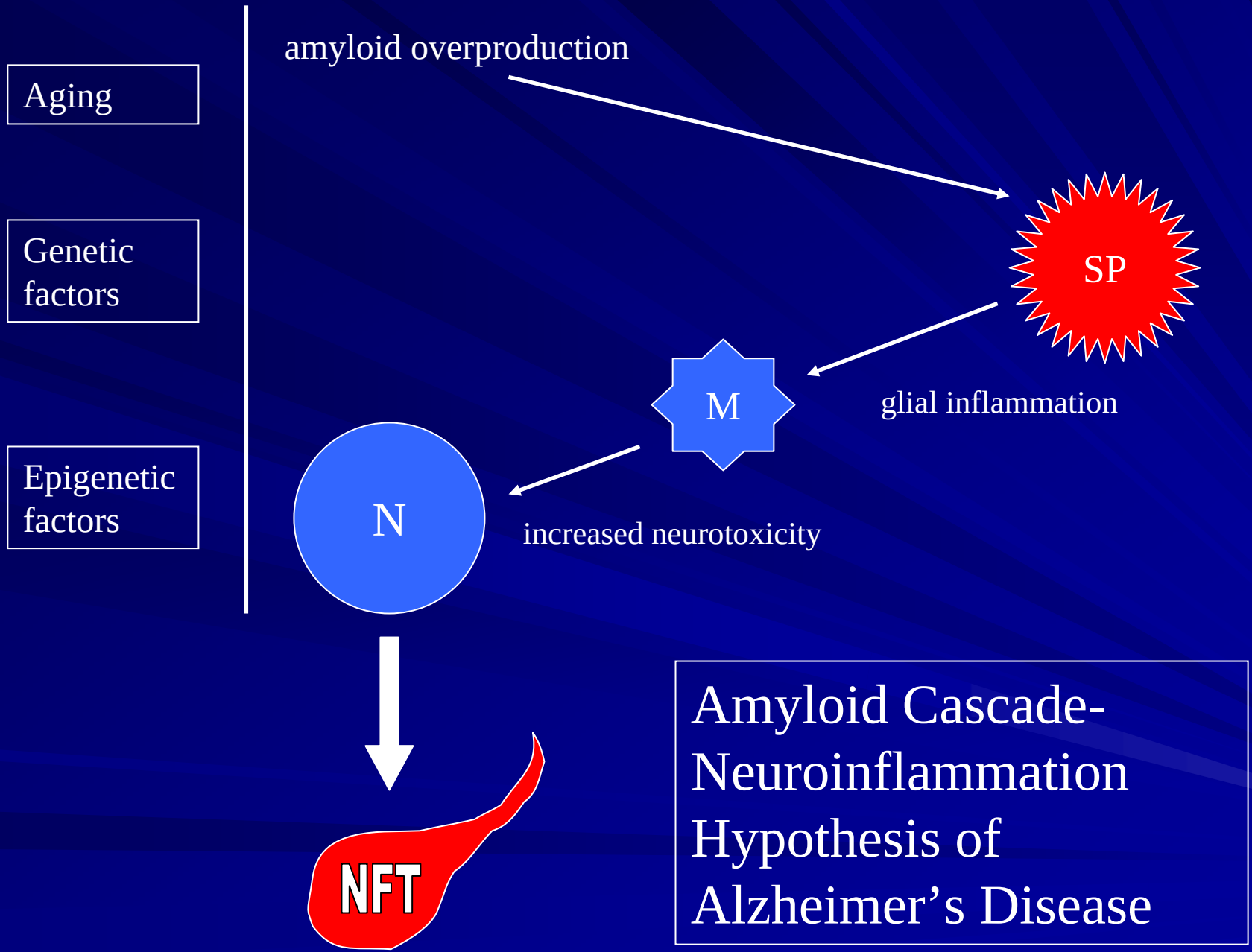
Acutely isolated microglia using FACS from

A - young (3-months-old) and **B** - aged (30-months-old) rats were subjected to flow-FISH analysis for telomere length determination.

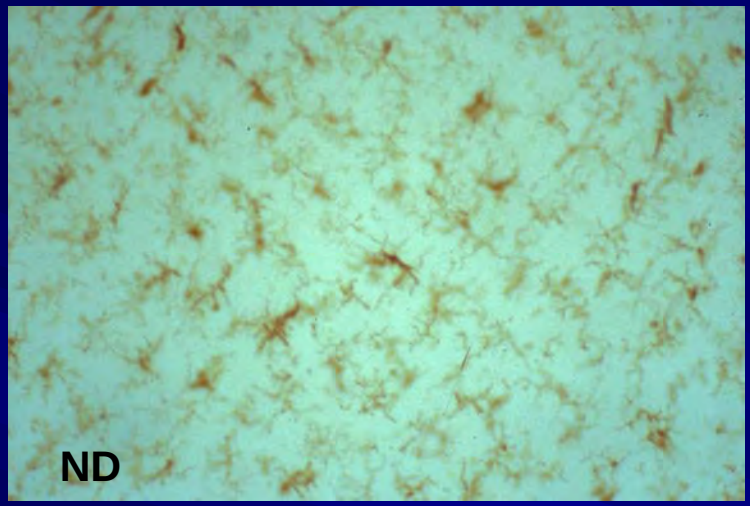


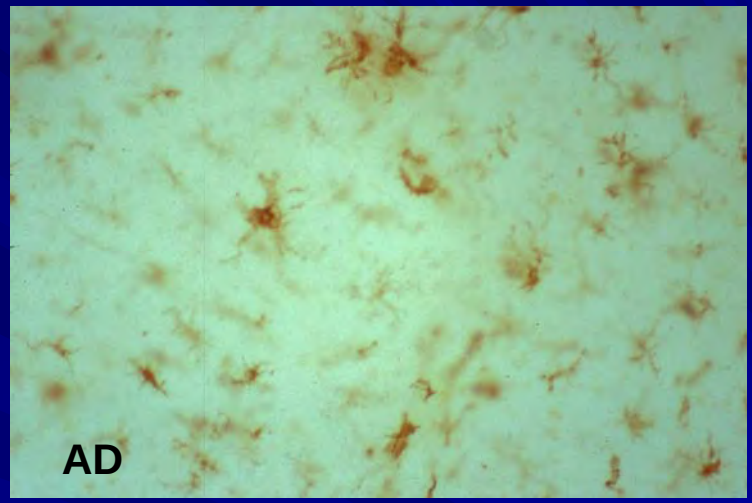
The role of microglia in Alzheimer’s disease

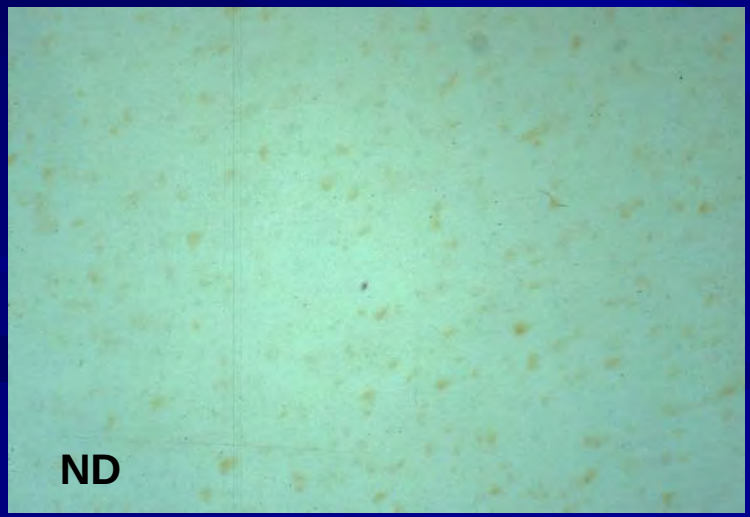
Old age and presence of intracerebral amyloid are major risk factors for AD

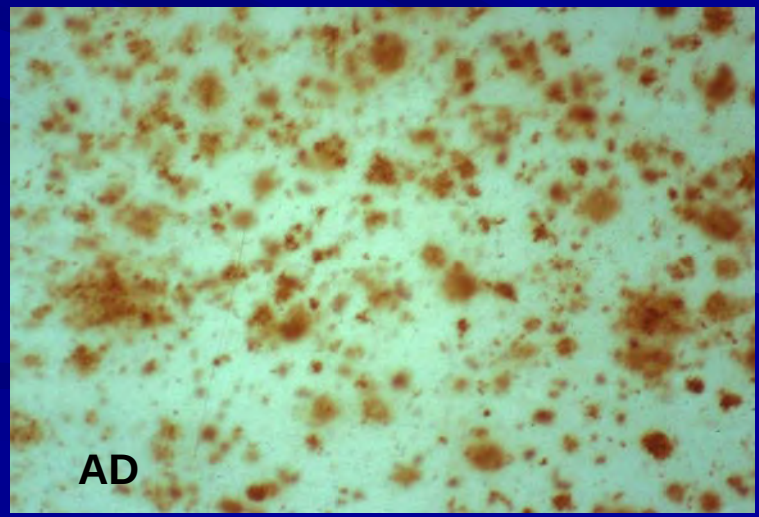

Aging
Genetic factors
Epigenetic factors
amyloid overproduction
SP
glial inflammation
M
N
increased neurotoxicity
NFT
Amyloid Cascade-Neuroinflammation Hypothesis of Alzheimer's Disease

Comparison of microglial activation with amyloid deposition

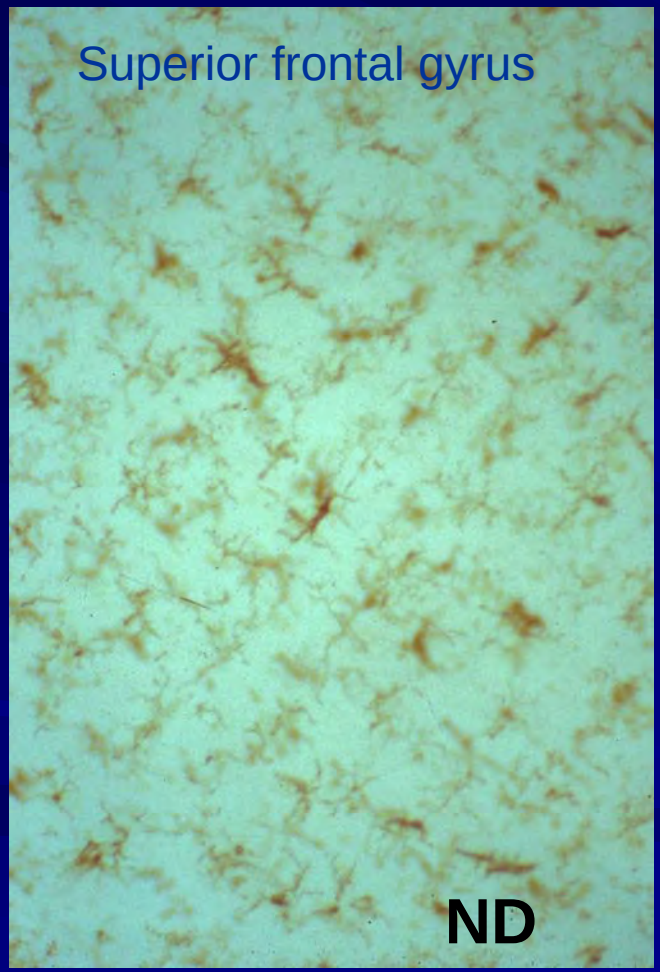


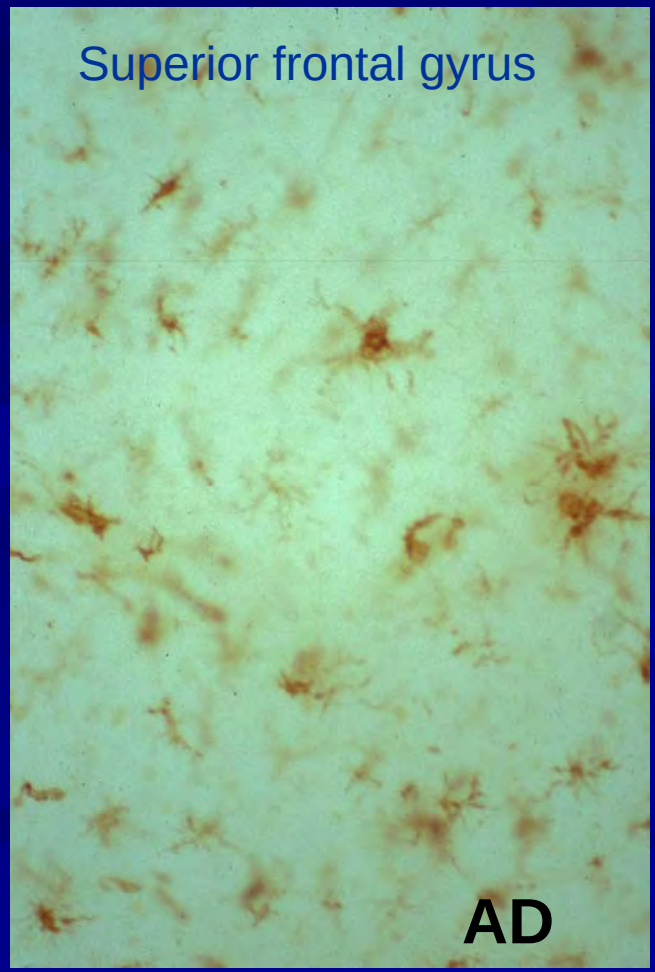




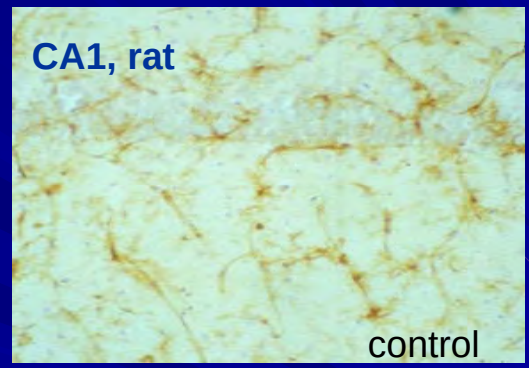


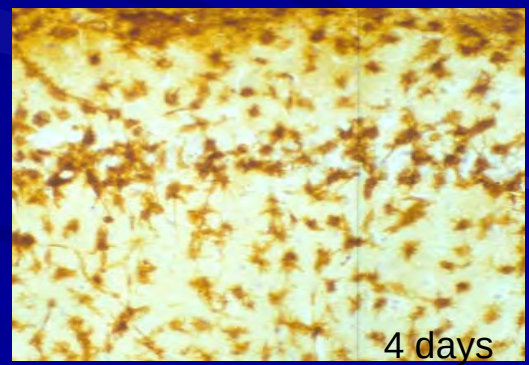
Comparison of microglia in AD and ND brain (LN-3 immunohistochemistry)



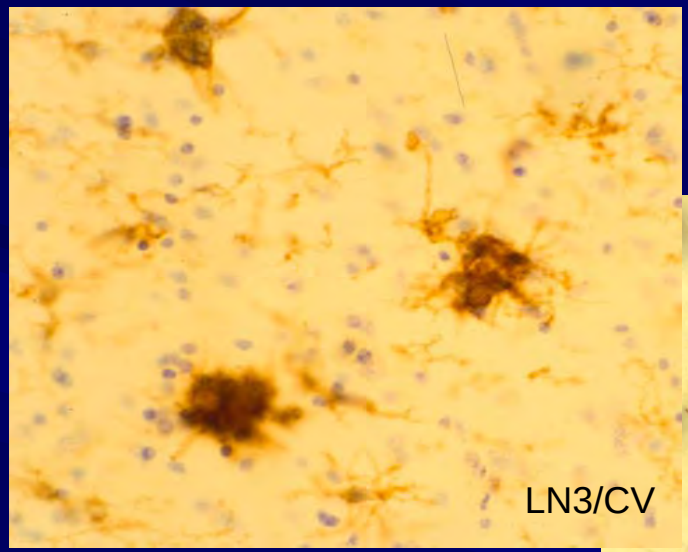


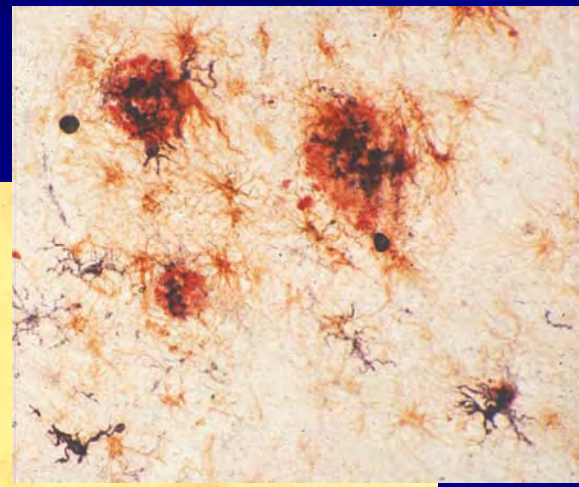
Compare that to acute microglial activation during ischemia:

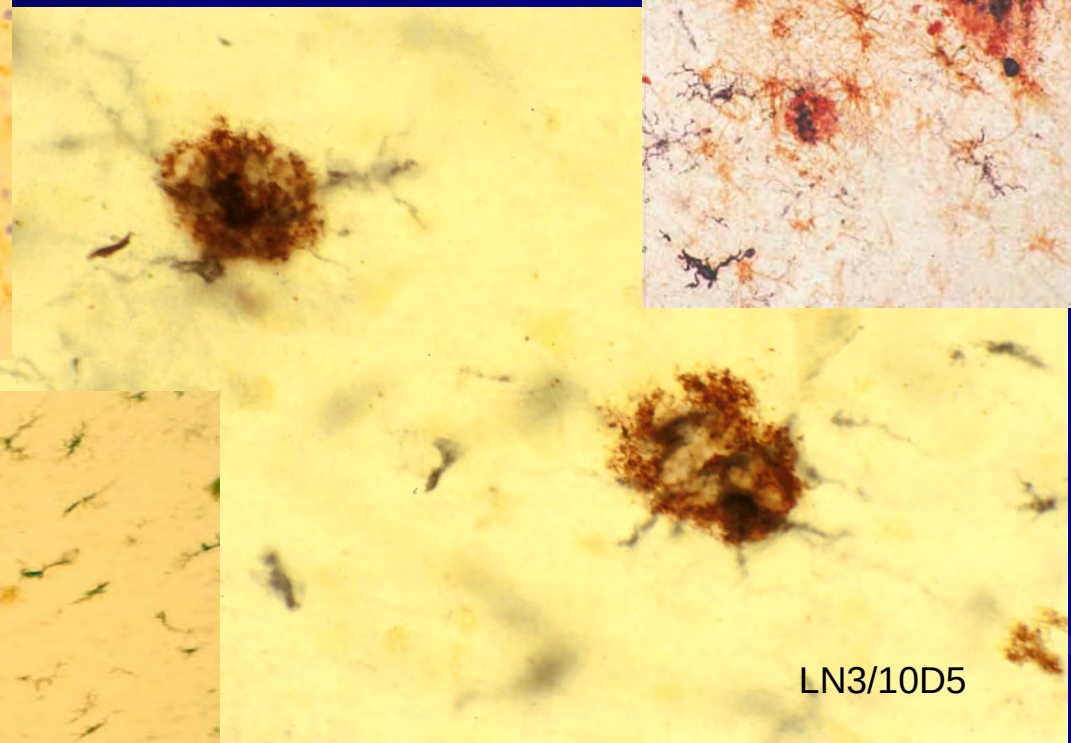


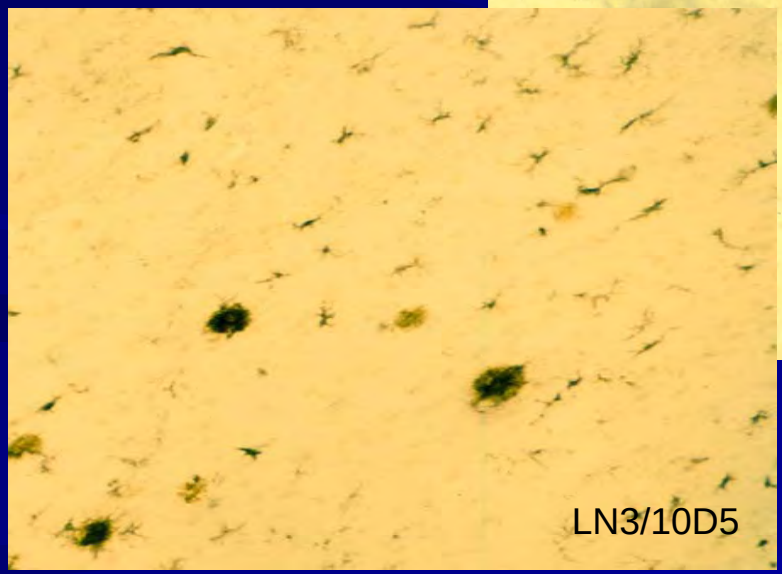


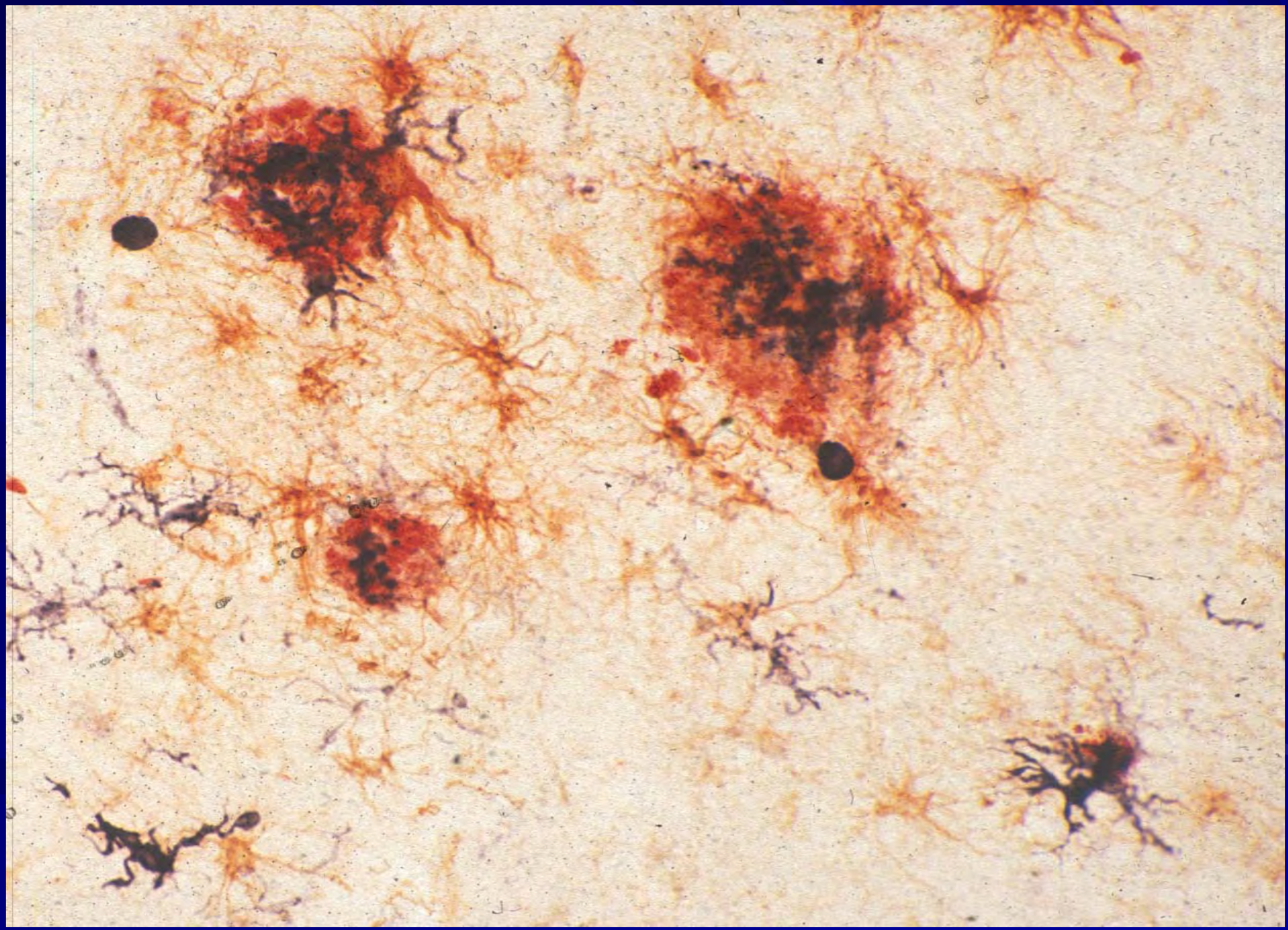
Presence of extracellular amyloid deposits causes clustering of microglia



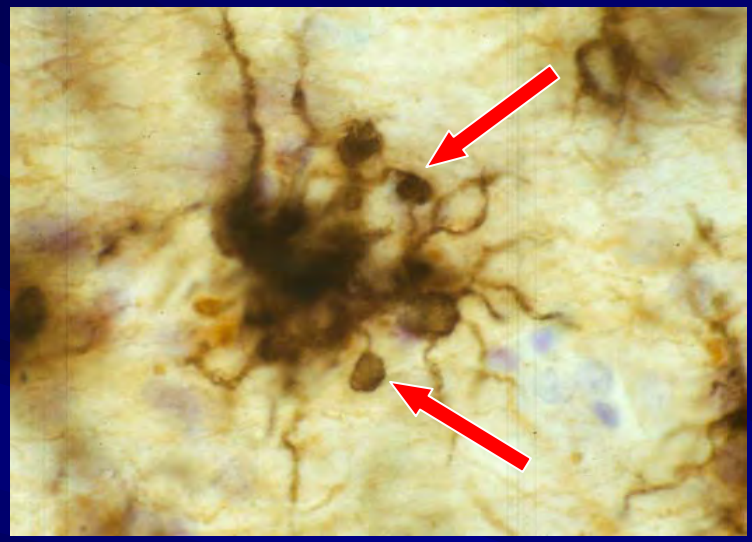


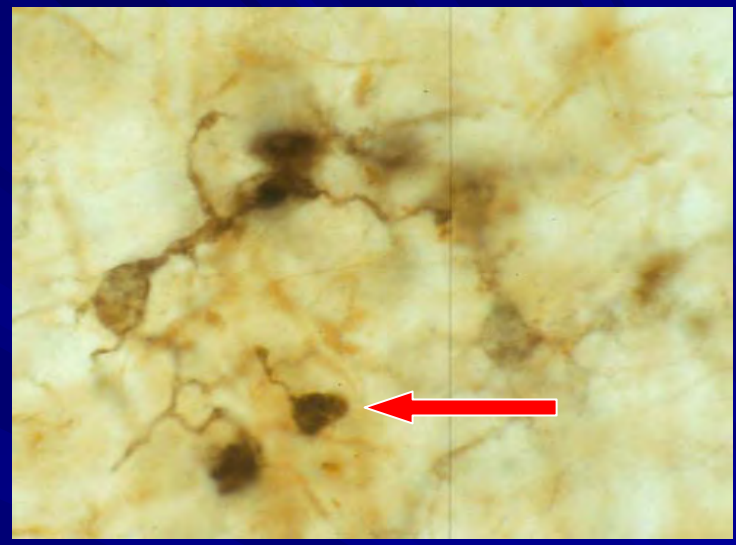


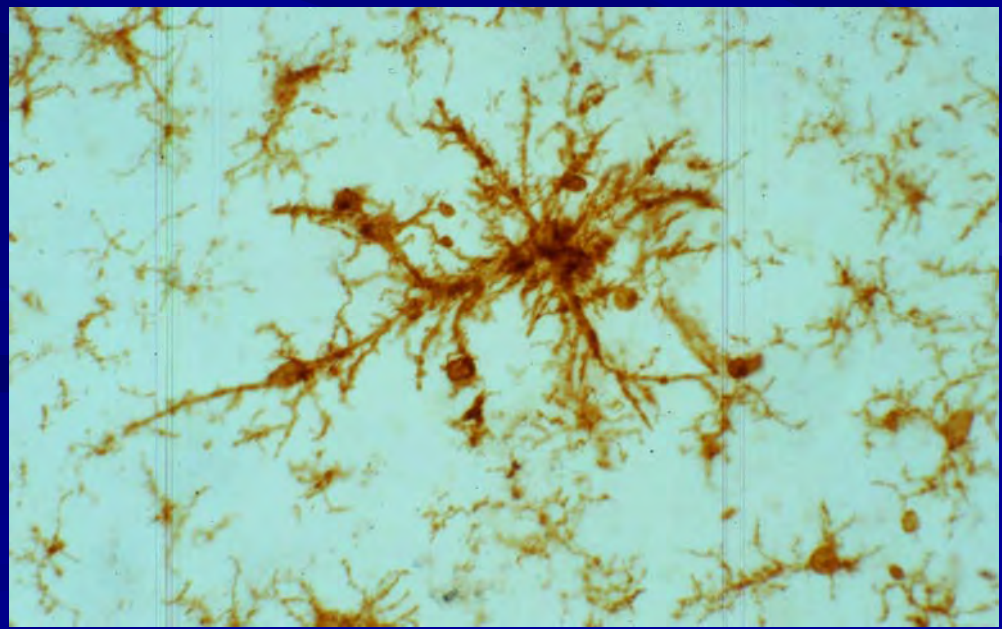




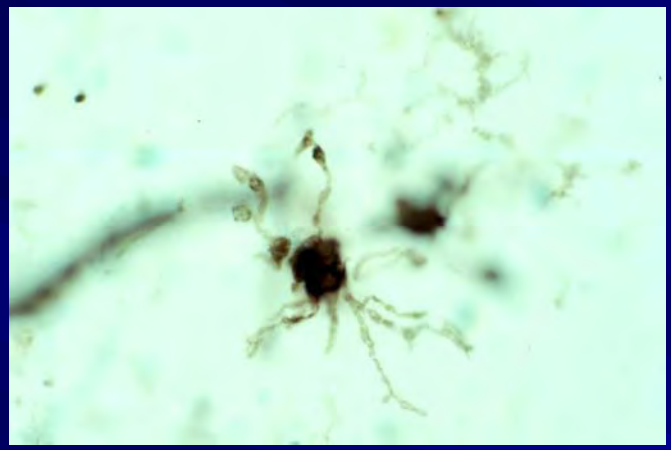
Microglial spheroids and fusions

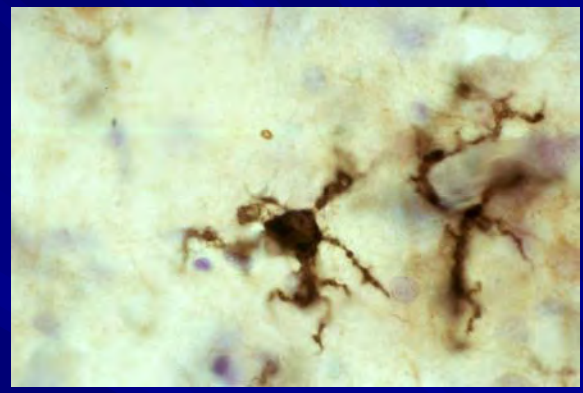


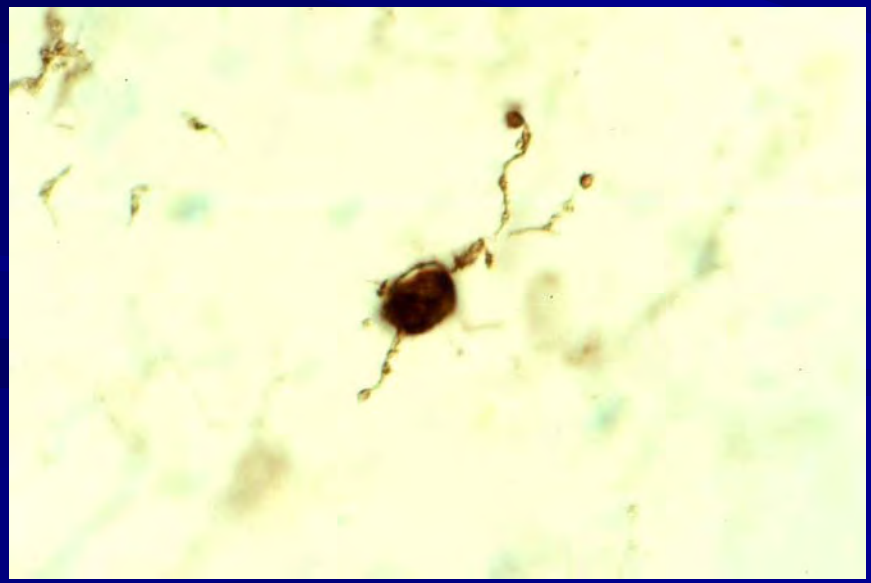


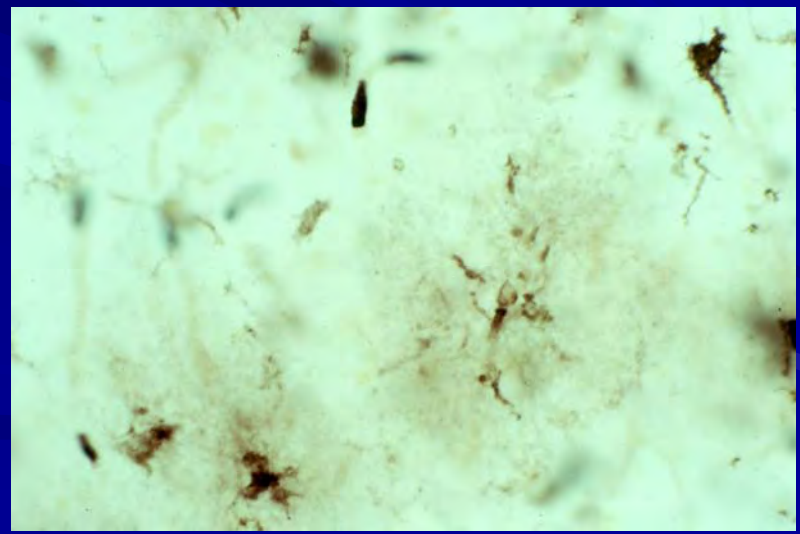


Other microglial abnormalities indicate that there is widespread microglial degeneration in AD brain.

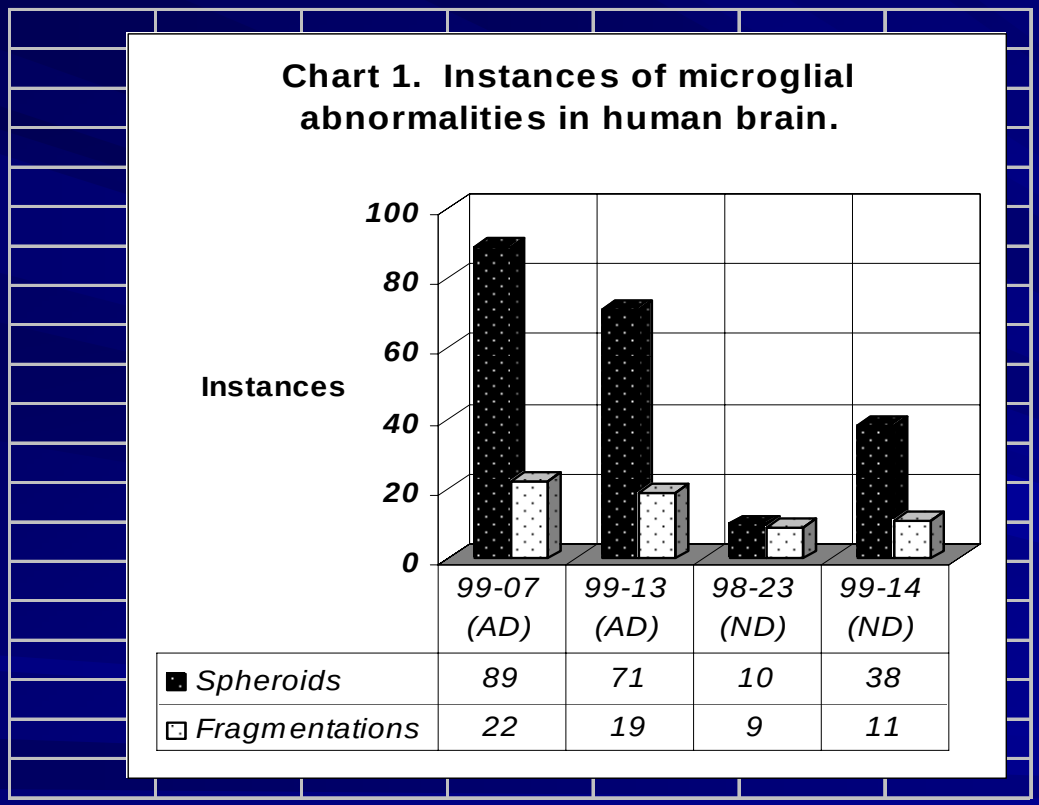


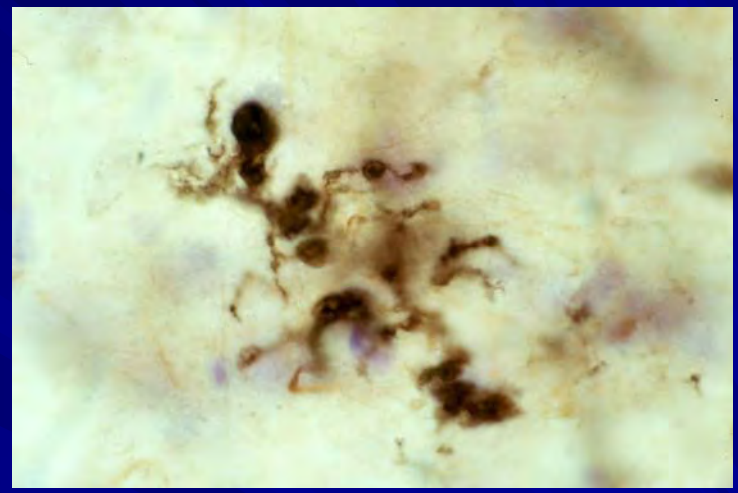


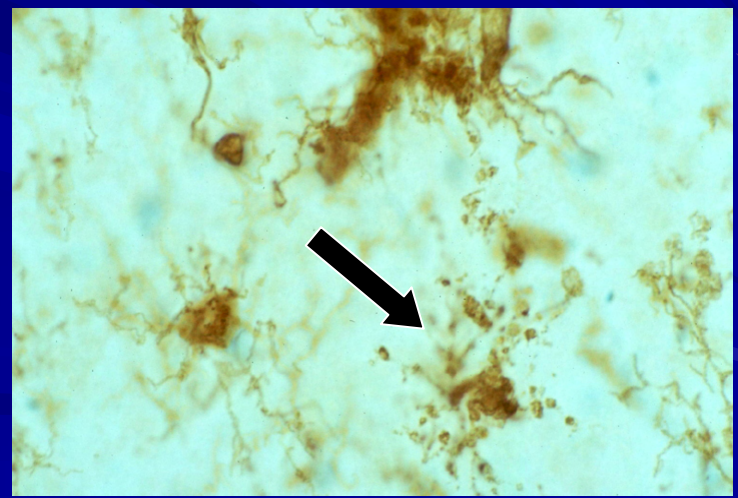




Dystrophic microglia are more numerous in AD brain.

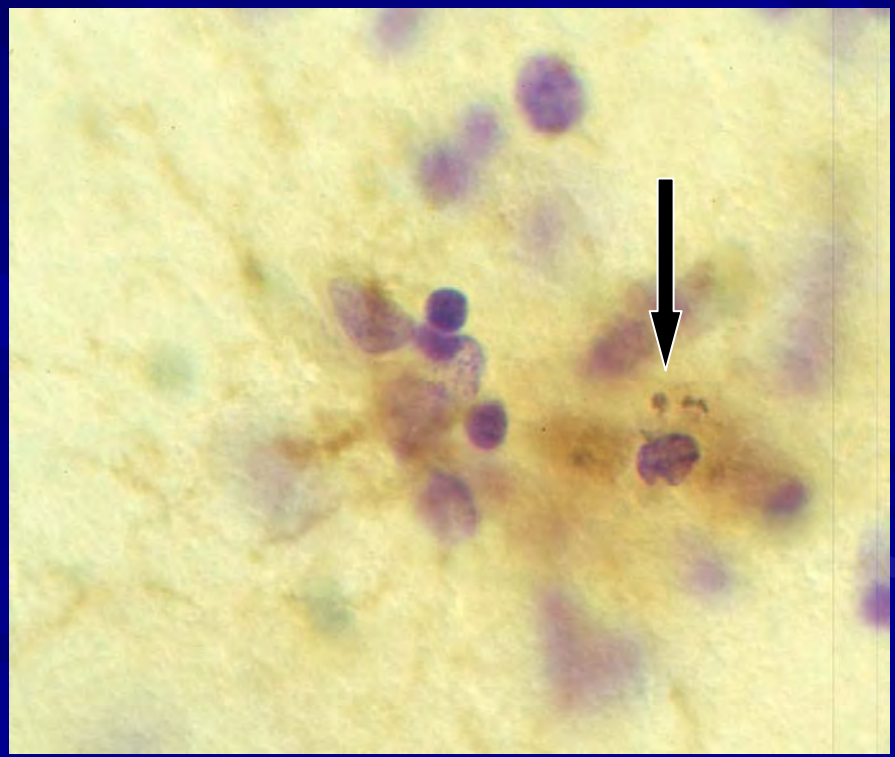




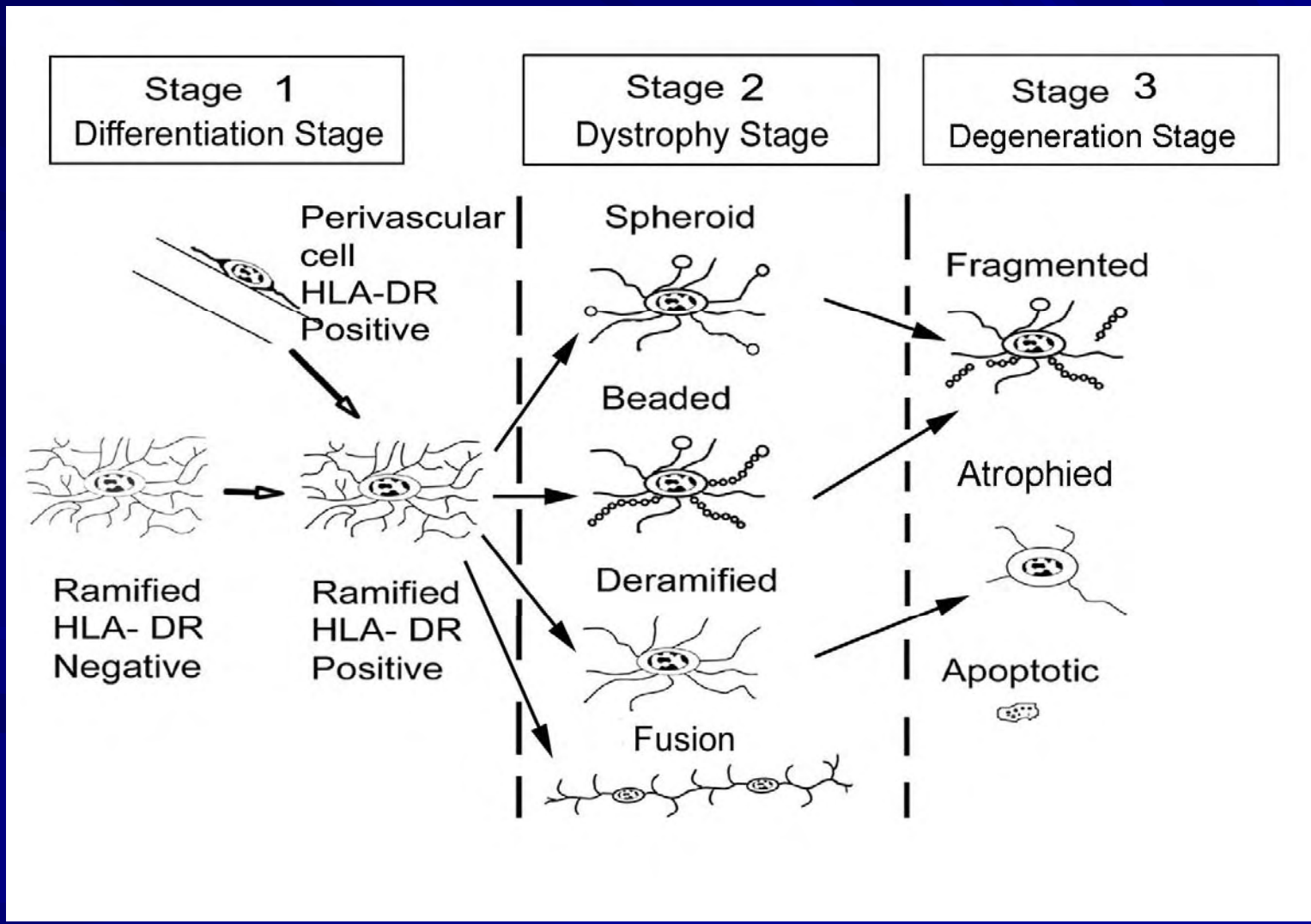


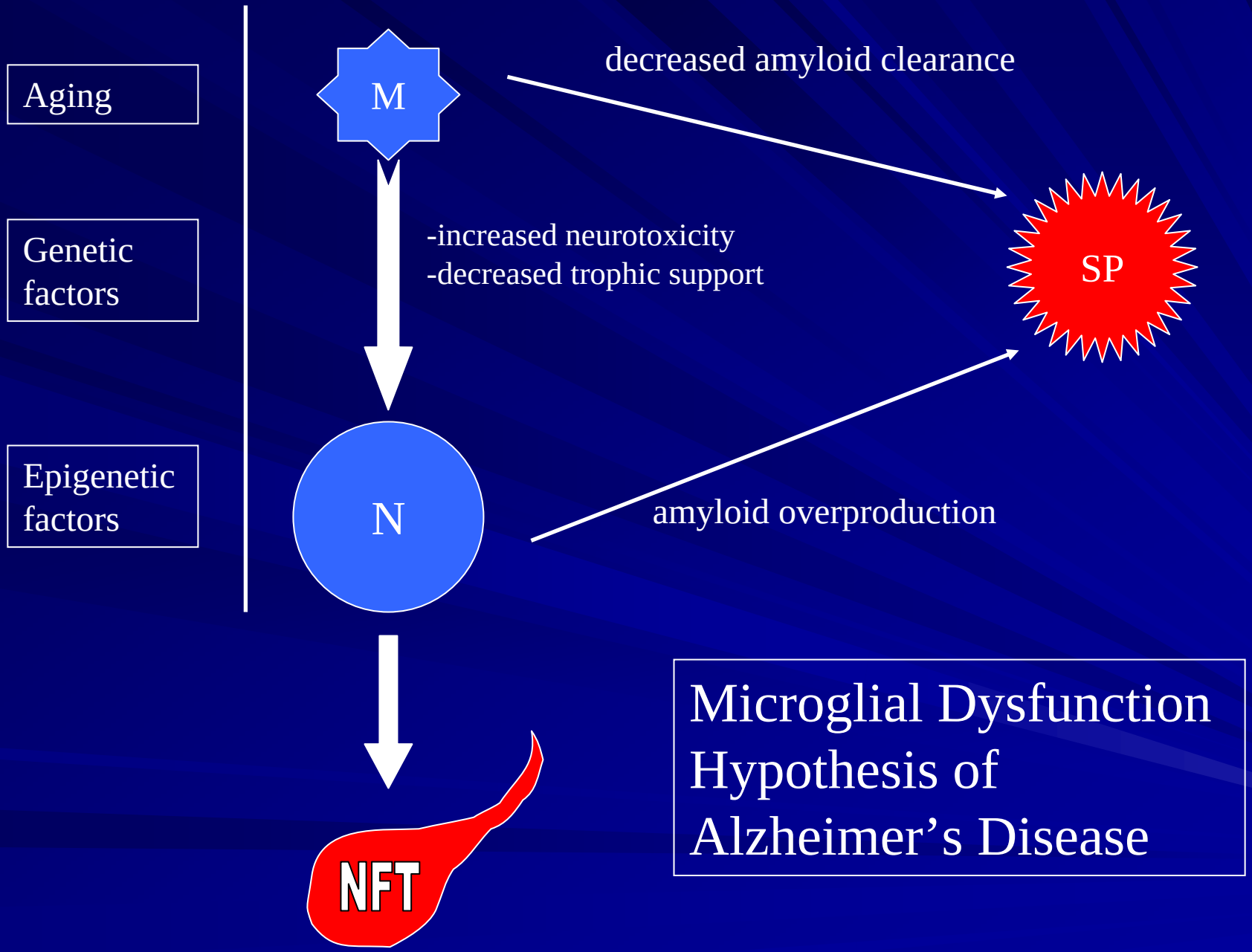
There is microglial apoptosis in AD.

- Lassmann et al., Acta Neuropathol. 89:35-41, 1995.
- Yang et al., Am J Pathol 152:379-389, 1998.
- Jellinger and Stadelmann, J Neural Transm Suppl (60):21-36, 2000



Microglial degeneration may occur in distinct stages




Aging
Genetic factors
Epigenetic factors
M
decreased amyloid clearance
-increased neurotoxicity
-decreased trophic support
SP
N
amyloid overproduction
NFT
Microglial Dysfunction Hypothesis of Alzheimer's Disease

What could account for microglial degeneration?

- Aging
- Amyloid
- Cell senescence

Acknowledgements

- Barry Flanary, Ph.D.
- Georg Kreutzberg, M.D.
- Manuel Graeber, M.D.
- Joseph Rogers, Ph.D.
- Larry Sparks, Ph.D.
- Nicole Sammons, B.S.
- Amanda Kuhns, B.S.

McKnight Brain Research Foundation, University of Florida

Institute for the Study of Aging

National Institutes of Health